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Cytological studies of certain apple varieties and their seedlings, with special reference to their value as stocks

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CYTOLOGICAL STUDIES OF CERTAIN APPLE VARIETIES AND THEIR SEEDLINGS WITH SPECIAL REFERENCE TO THEIR VALUE AS STOCKS

BY

Samuel Wheeler Edgecombe

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Major Subject Horticulture

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In charge of Major work

Signature was redacted for privacy.

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Dean of Graduate College

Iowa State College
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INTRODUCTION

American nurserymen have depended largely upon the so-called French Crab, either American or French grown, for their seedling apple stocks although some Vermont and Northern grown seedlings have been used. Apple stocks are now being produced in considerable quantities by Oregon and Washington nurserymen. None of these seedling stocks are entirely satisfactory in the upper Mississippi Valley because they lack sufficient hardiness to withstand the severe winter temperatures of that region.

The Pomology Subsection, Iowa State College, Ames, Iowa, is making efforts to find stocks which will be more satisfactory than those now used in the upper Mississippi Valley. The three lines of approach which are being followed are: (1) The development of own-rooted stocks, (2) Stocks grown from open-pollinated seeds from selected parents and, (3) Double worked stocks in which an intermediate stem piece is inserted between the original root stock and the top-worked variety. (Maney 25, 26, 27, 28, and 29).

Certain vigorous hardy varieties, such as, Hibernal and Virginia Crab, when used as seed parents in the Iowa studies, produced open-pollinated seedlings which were so lacking in vigor that they were without value for stocks. (Maney 26 and

27). On the other hand, varieties such as, Whitney and Ames 550[1], which are medium vigorous varieties, produced open-pollinated seedlings, which were very vigorous and excellent for stock purposes.

After Maney (27) observed the striking behaviour of these four varieties, he grew open-pollinated seedlings of them for four successive seasons. The seed was collected from various locations, so that each variety was likely to have been pollinated by a wide range of pollenizers. However, regardless of the year or place of collection Maney (28) found that the two varieties, Hibernal and Virginia Crab, produced seedlings lacking in vigor while the other two varieties, Whitney and Ames 550, produced seedlings which were strong and vigorous. (Also see figures 1-3).

Further, in his breeding investigations, Lantz (20) of the same station, has had great difficulty in making crosses using Hibernal. (Unpublished data). When Hibernal was used as the pollen parent, the crosses were complete failures. When used as the pistillate parent an occasional fruit was obtained; but such fruits usually had very few seeds. These seeds germinated poorly and finally the few resulting seed-

[1] Ames 550 is a seedling produced by the Pomology Subsection, Iowa State College, Ames, Iowa, from the cross, Briar Sweet x Mercer County Crab.

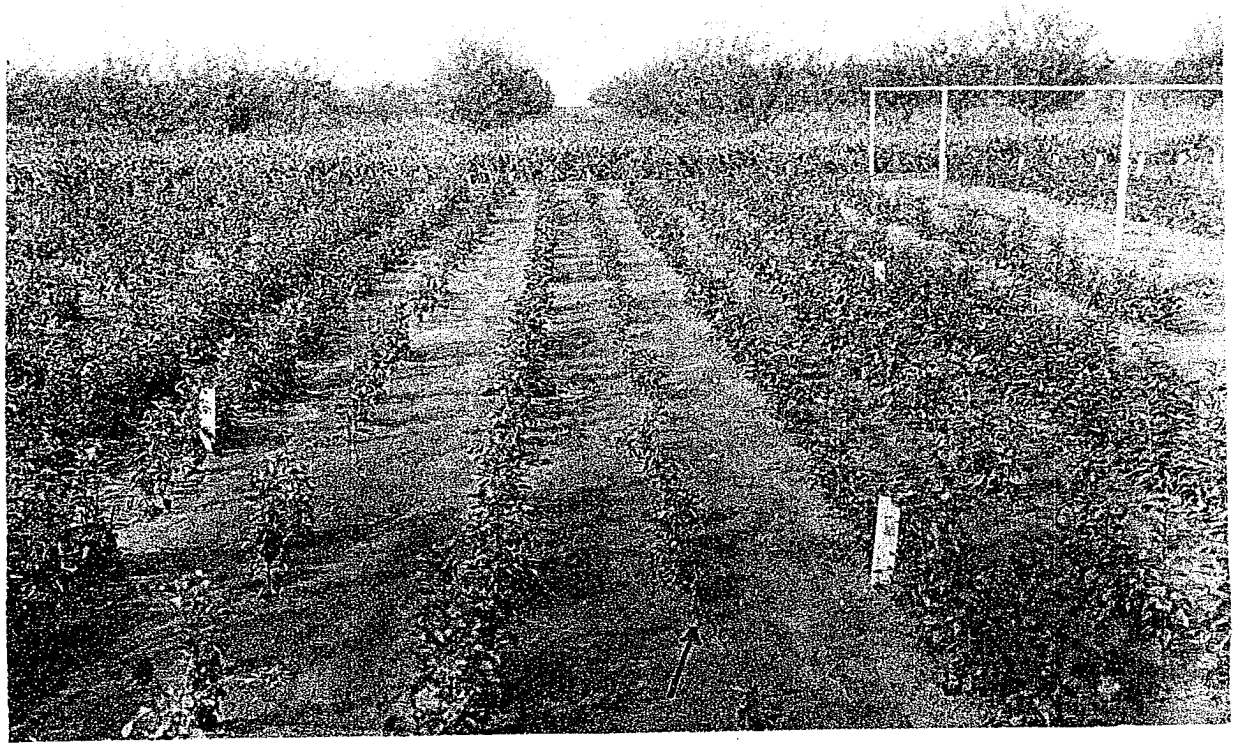


Figure 1. The apple seedlings in the row indicated by the arrow are characteristic of one year old Virginia Crab seedlings. Note the poor stand and lack of vigor of this row of seedlings as compared with the seedlings in the adjoining rows.

Figure 2. The row indicated by the arrow of one year old Hibernal seedlings show the type of stand and vigor which may be expected when Hibernal is used as a seed parent.

Figure 3. Comparison of five year old open-pollinated seedlings of Virginia Crab, Ames 550, and French Crab. The Virginia Crab seedlings are in the row indicated by the arrow, the seedlings in the row on the left are Ames 550, and those on the right are French Crab seedlings.

Table 1. SUMMARY OF ANTONOVKA AND HIBERNAL PROGENIES IN THE SEEDBED SHOWING NUMBER OF SEEDS PLANTED, NUMBER AND PERCENTAGE OF SEEDS GERMINATING, NUMBER OF SEEDLINGS LIVING AT THE END OF THE FIRST YEAR'S GROWTH, AND THE AVERAGE HEIGHT OF THE SEEDLINGS IN EACH PROGENY AT THE END OF THE FIRST YEAR'S GROWTH.

Breeding Number	Parentage	No. seeds planted	Germination results		Average ht. of progeny in inches
			Number	Percentage	
14210	Antonovka x Delicious	375	271	72.27	9.88
14246	" x Delicious	87	64	73.56	7.38
14212	" x Black Oxford	238	200	84.03	9.17
14208	" x Black Oxford	27	12	44.40	7.90
14211	" x Ashton	200	111	55.50	8.33
14240	" x Grimes	105	63	60.00	7.08
14213	" x Jonathan	115	84	73.04	6.12
14215	" x King David	182	159	87.36	8.39
14304	Hibernal x Ashton	29	13	44.83	5.58
14221	" x Black Oxford	106	55	51.88	3.60
14218	" x Delicious	73	30	41.10	4.00
14223	" x King David	113	23	20.36	3.31
14222	" x Northern Spy	27	4	15.00	4.00
14234	" x Delicious	20	5	25.00	4.40
14318	" x Delicious	5	1	20.00	5.00
14224	" x Jonathan	3	2	66.67*	0.00*
14322	" x Delicious	37	14	37.83*	0.00*
14237	" x Jonathan	11	0	0.00	0.00

*The average height of these seedlings was measured at the end of the first year's growth. By that time all of the seedlings in these progenies were dead. These data are according to Lantz (20).

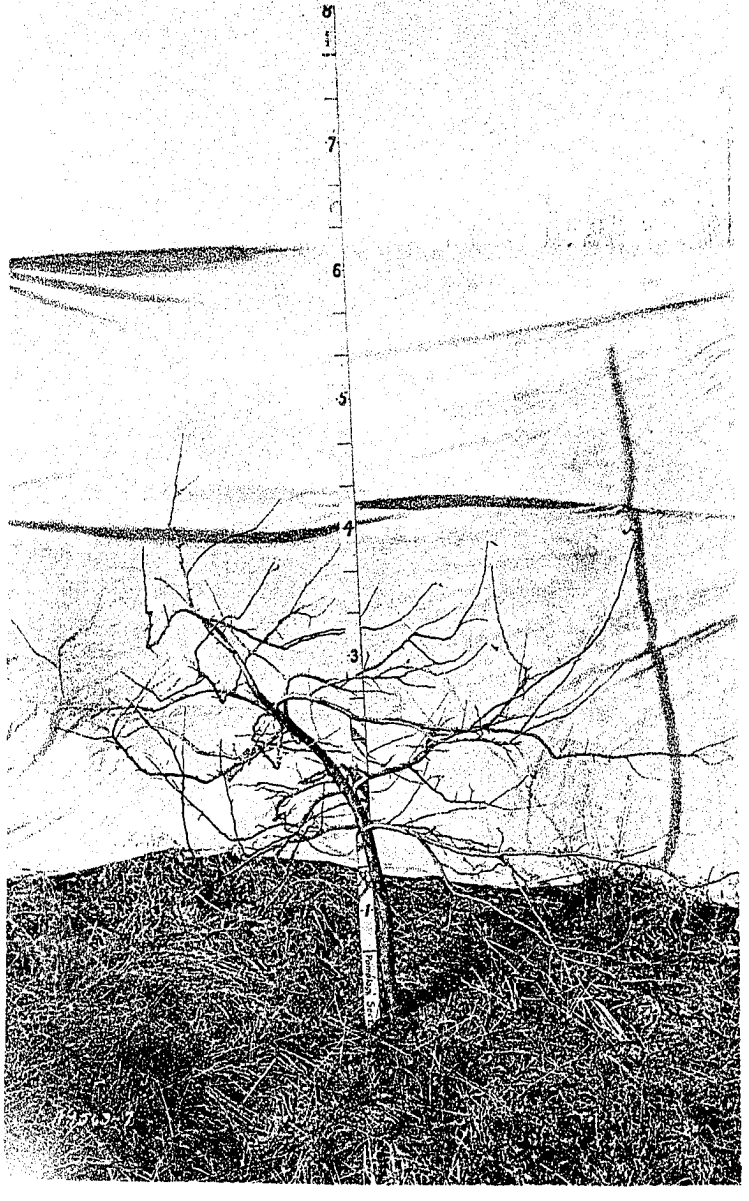


Figure 4. Hibernal x Ashton. (3n x 2n). This cross-bred apple seedling is eleven years from seed.

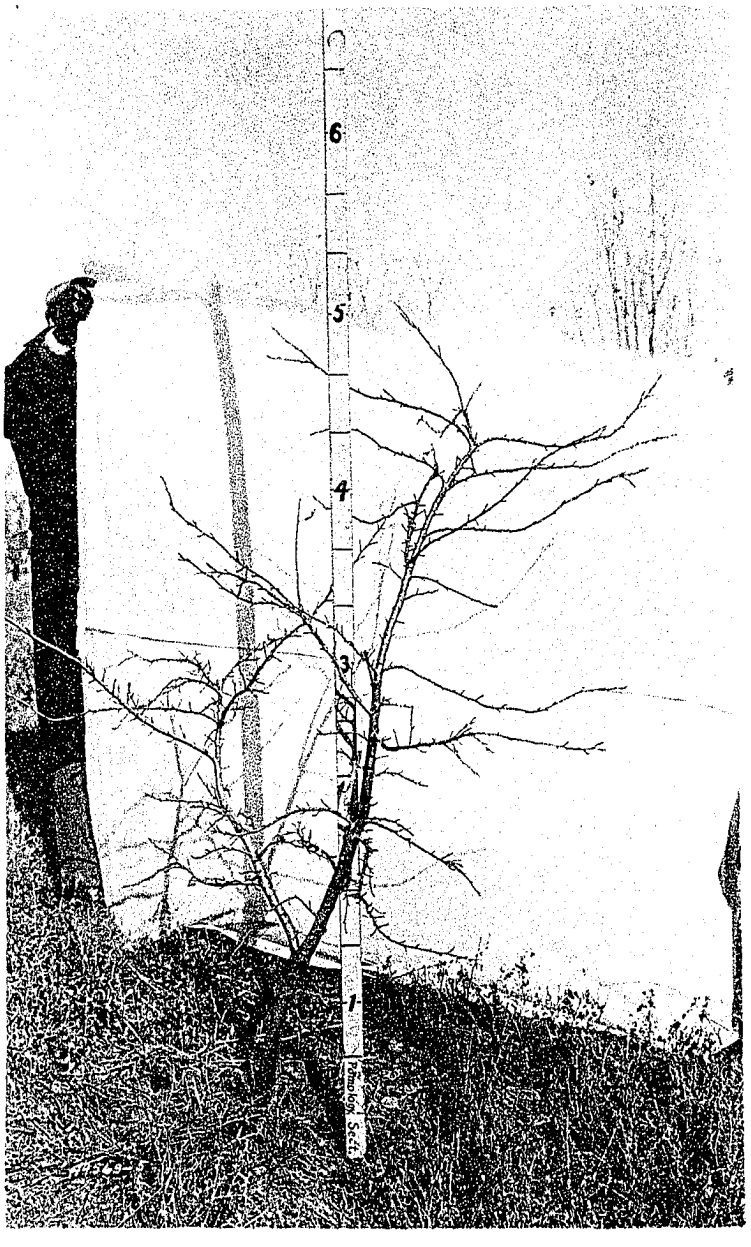


Figure 5. Hibernal x Ashton. (3n x 2n?). This crossbred apple seedling is eleven years from seed.

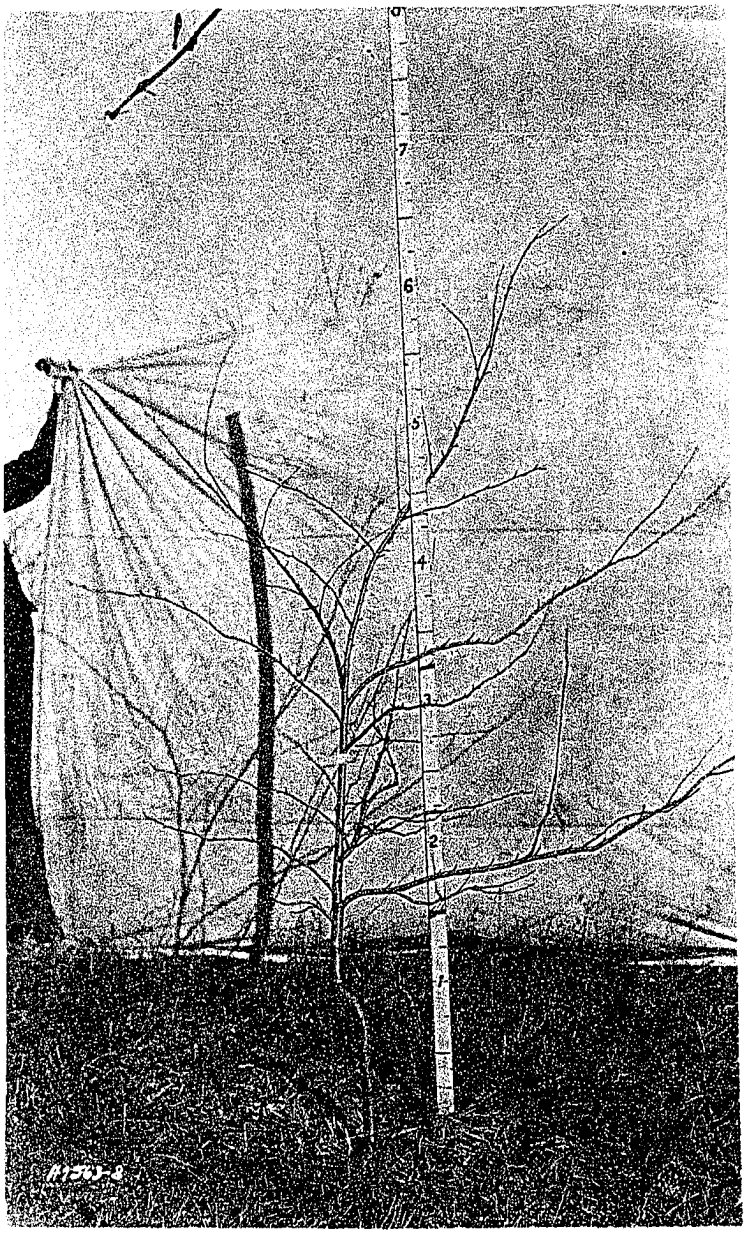


Figure 6. Hibernal x King David. (3n x 2n).
This crossbred apple seedling is thirteen years from seed.

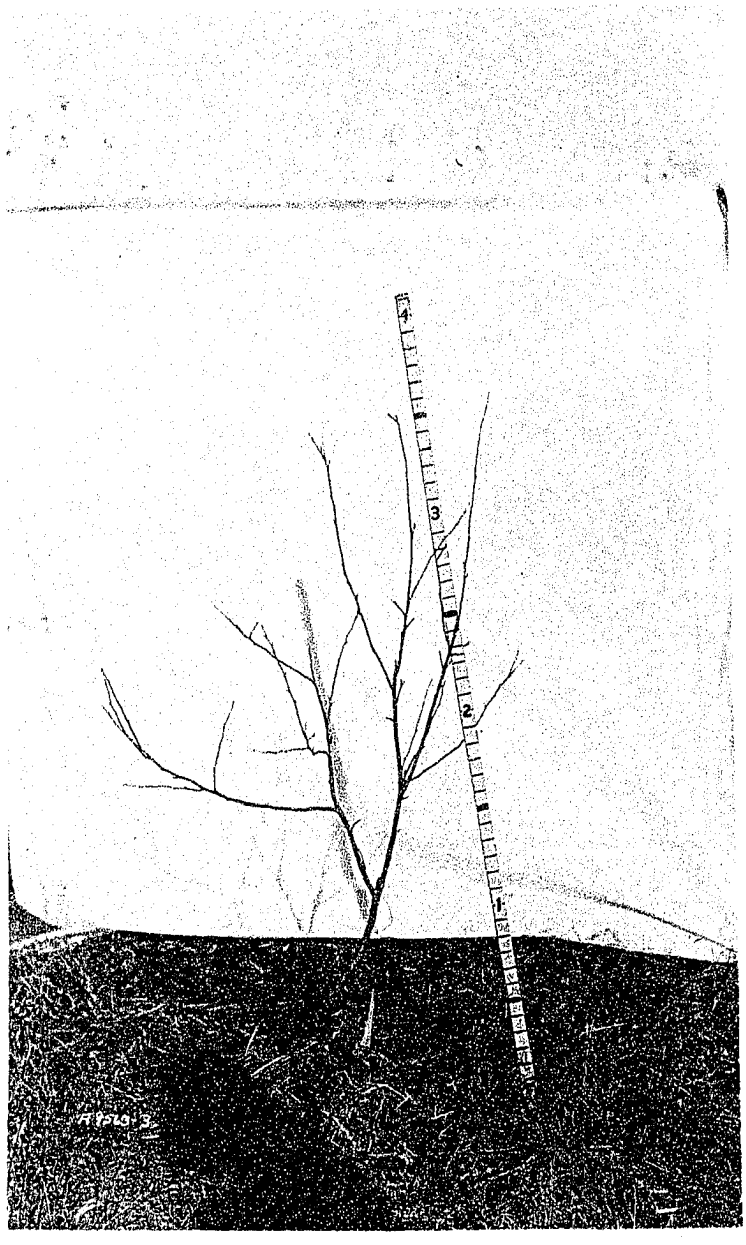


Figure 7. Hibernal x Black Oxford. (3n x 2n?).
This crossbred apple seedling is thirteen years from seed.

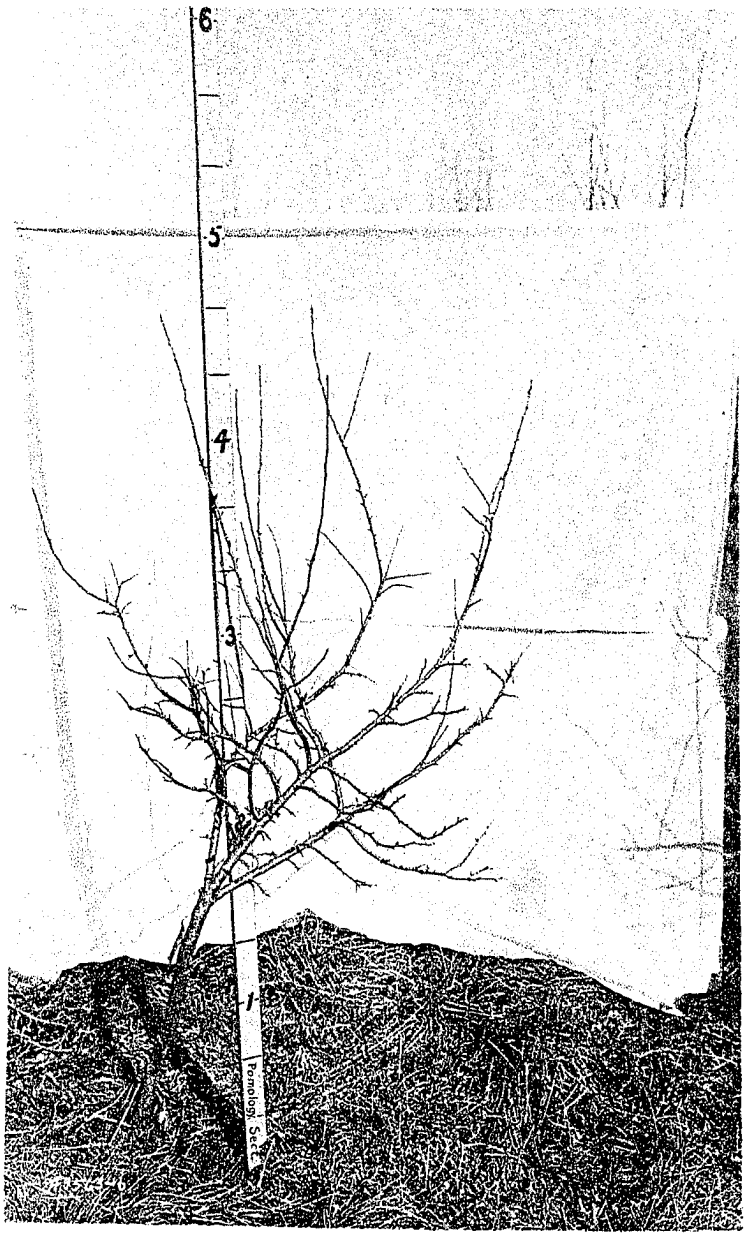


Figure 8. Hibernal x Jonathan. (3n x 2n).
This crossbred apple seedling is eleven years from seed.

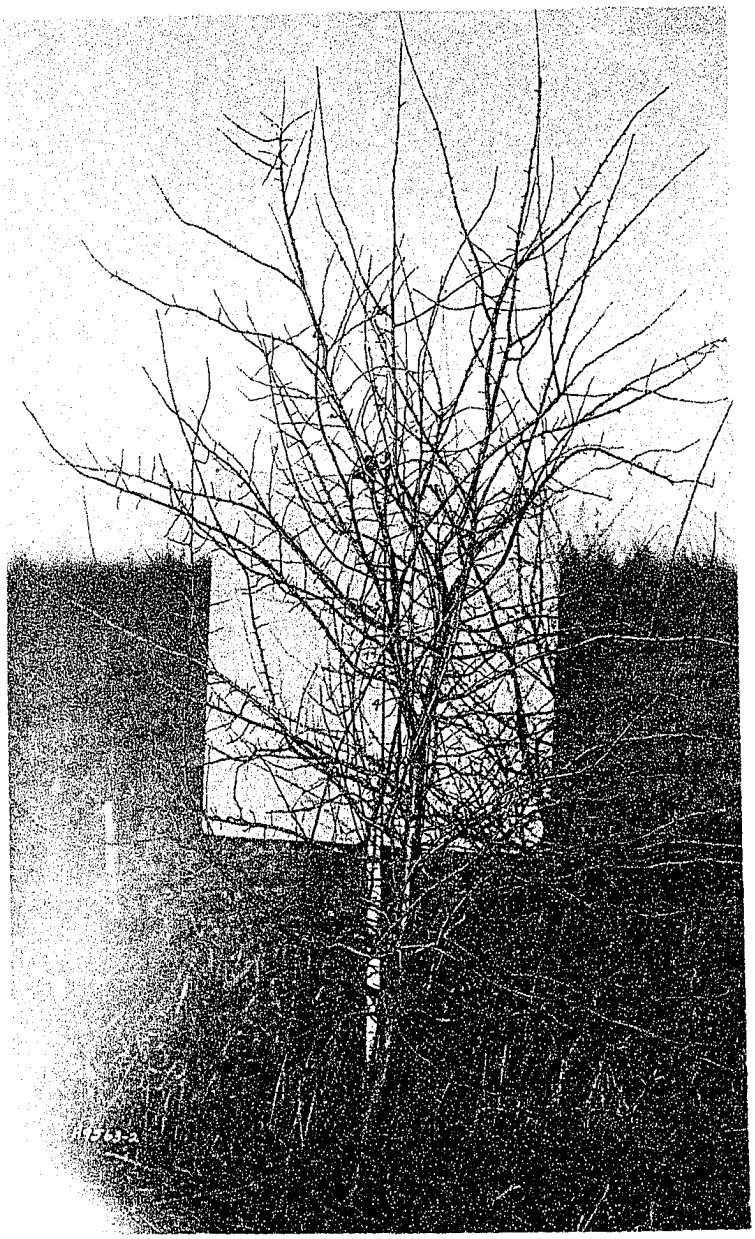


Figure 9. Hibernal x Delicious. (3n x 2n).
This crossbred apple seedling is ten years from seed.

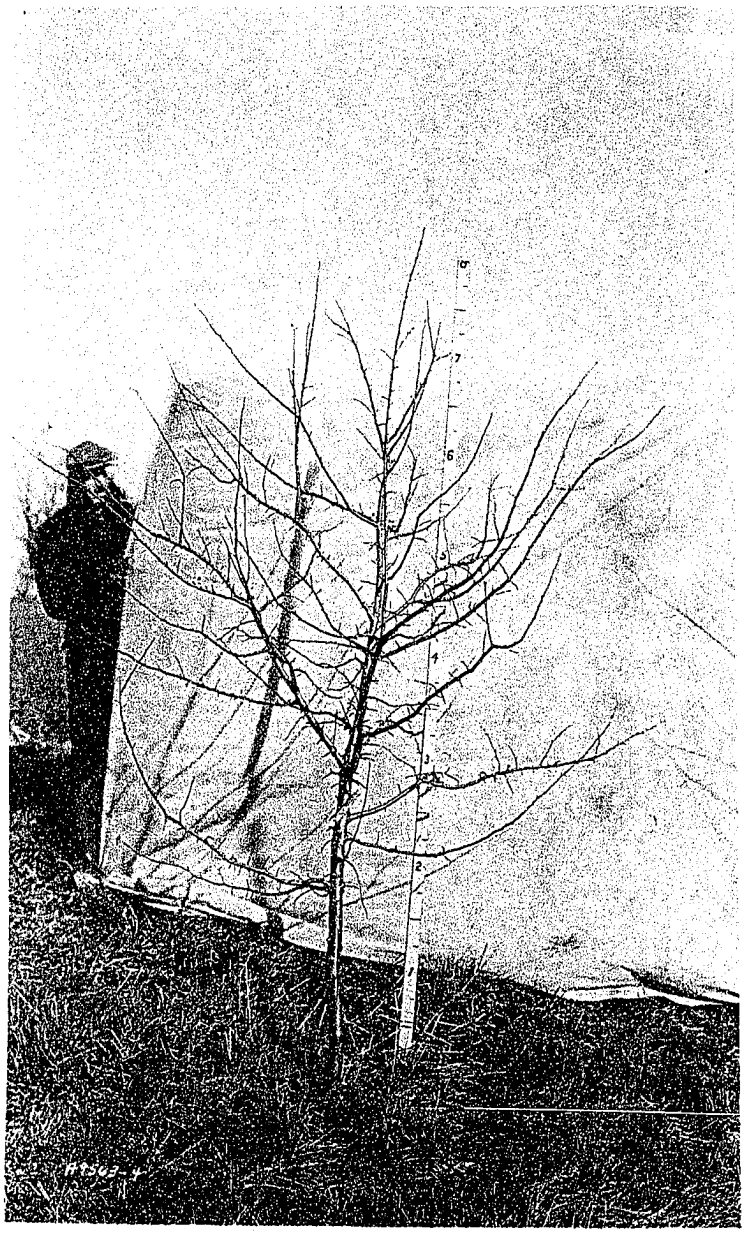


Figure 10. Hibernal x Esopus. (3n x 2n).
This crossbred apple seedling is ten years from seed.

lings were always lacking in vigor throughout their lives. In fact, only rarely have they reached fruiting age or size. (See figs. 4-10 and Table I).

Other workers, Beaumont (3) and the experimental farm at Lennoxville, Quebec (23) have reported that Hibernal has produced seedlings of desirable vigor. This apparently conflicting evidence should not be taken as such, because Gibb (11, 12) and Lyon (24) have shown that when the various Russian apples were introduced into America, several strains were named Hibernal. In view of the diametrically opposite results observed it is possible that the clones used by the different investigators are not the same.

Until 1930, the multiple factor hypothesis was used to explain the lack or presence of desirable vigor in seedlings of apple varieties. At that time Crane and Lawrence (4) working in close co-operation with Darlington and Moffett (7) advanced another explanation for such cases of extreme lack of vigor as were observed by Maney (28) with the seedlings of Hibernal and Virginia Crab. They had observed a variety, Bramely's Seedling, which behaved in much the same manner as Hibernal and Virginia Crab, in that it produced seedlings lacking in vigor. This variety was investigated cytologically by Darlington and Moffett (7) and found to be a triploid form with 51 chromosomes. Furthermore, 13 open-pollinated

seedlings of Bramely's Seedling were examined cytologically. All were aneuploids with chromosome counts ranging from 38 for the lowest to 47 for the highest in the series. On the basis of these data they advanced the theory that crosses between triploid and diploid apple varieties yield only aneuploid seedlings, which are lacking in vigor due to their aneuploid chromosome constitution.

Crane and Lawrence (4) at the same time reported the results of nine crosses involving diploid x diploid, diploid x triploid and triploid x triploid apple varieties. Only seedling vigor observations were made but the evidence tended to confirm Darlington and Moffett's theory. Also, results obtained by Dahl (6) were in agreement with the theory. Up to the time of the initiation of this investigation this was the situation and since the entire theory was established upon cytological evidence involving only 13 open-pollinated seedlings of the variety, Bramely's Seedling, it seemed that more cytological evidence on vigor in exceptional weak seedlings would be very valuable.

PURPOSE OF THE INVESTIGATION

The purpose of this investigation was to determine why varieties, such as Hibernal and Virginia Crab, produce weak and undesirable seedlings and why varieties such as Whitney and Ames 550 produce strong and desirable seedlings. In pursuit of this purpose the investigator undertook;

1, to determine the chromosome count and pollen formation in Hibernal, Virginia Crab, Whitney, Ames 550, and any others which might assist in the development of a more complete understanding of the behavior of the first four varieties as seedling producers;

2, to determine whether Hibernal, Virginia Crab, Whitney and Ames 550 would set fruit in reciprocal crosses with diploid and triploid varieties;

3, to germinate the seeds resulting from these crosses and ascertain the chromosome number of the resulting seedlings in an effort to determine if vigor is associated with chromosome number as postulated by Darlington and Moffett (7).

REVIEW OF LITERATURE

Kobel (15) conducted the first cytological investigation on the apple. In a series of papers, he published counts on 28 horticultural varieties and 10 species (16, 17, and 18). He found that the varieties were divided into two groups: namely, (1) Varieties with normal reduction division, and (2) varieties with abnormal reduction division. Thirteen of the investigated varieties belonged to the first group and fifteen belonged to the second group. The 2n chromosome count of the varieties belonging to the first group was 34, while in those varieties belonging to the second group it varied from 36 for some to as high as 51 in others. All the species with the exception of Malus Halliana belonged to the first group and had an n chromosome number of 17. Malus Halliana had a 2n count of 47-49 and was irregular in its reduction division.

Shortly after Kobel (16) published his first work, Shoemaker (46) found that normal pollen development in the variety, Delicious, is associated with its ability to function satisfactorily either as a pollenizer or as a parent in fruit breeding or stock investigations. In contrast, he showed that the variety Stayman Winesap, which has abnormal pollen development, is lacking in ability to function satisfactorily as a parent in breeding or stock investigations. He stated that Delicious has 14 pairs of chromosomes and that Stayman

Winesap has more than 28 chromosomes in the 2n form.

About the same time, Rybin (41) published on nine species and one horticultural variety. He found some of the species to be tetraploid (Malus Sargentii 64-69, M. Toringo 64-71, and Malus coronaria var. ioensis C. K. Schneid 65 chromosomes). Seedlings of these species were used in making the above counts. The one horticultural variety was a diploid (Tchulanovka).

Later, Rybin (42) investigated the somatic divisions in root tips of seedlings belonging to 30 varieties of the cultivated apple, as well as the reduction division in the anthers of nine varieties. He found that the somatic number was 34 in all varieties. Eight of the varieties had regular reduction division, normal pollen and a diploid number of 34 chromosomes. The ninth variety had irregular reduction divisions, abnormal pollen and a triploid chromosome number of 51. This was the first definite recorded case of triploidy in apples.

Maney and Welter (30) found that Malus ioensis had 14 pairs of chromosomes and that a Mercer County seedling had a variable number ranging from 13 to 15 pairs. They noticed many cases of abnormal development in the Mercer County seedling. These abnormalities took the form of lagging chromosomes on the equatorial plate, which later on formed nuclei of their

own. As a result, it was not unusual to find five or six microspores in the tetrad stage.

Chromosome counts for seven apple varieties were reported by Heilborn (13) in 1928. Six varieties were diploids (2n = 34) and one, Gravenstein, was reported to be aneuploid (2n = 45). However, it is probable that the last variety is a triploid since Kobel (18) found it to have 45-46 and Nebel (36) reported a somatic count of 51 for it and seven of its bud sports.

Nebel (34) investigated twenty-nine apples representing nine varieties and twenty species. He found the haploid complement in Malus was 17, and that diploid, triploid and tetraploid forms were present. Nebel was somewhat doubtful about the identity of the two species which he reported as triploid (Malus spectabilis and M. prunifolia). He was inclined to regard the trees from which he took his material as being wrongly named. It appears that his surmise was correct since Rybin (41) has reported both these species as diploid and Sax (44) has reported M. prunifolia as diploid.

In a later paper Nebel (36) reported 18 diploids and thirteen triploids. Among the triploids were seven sports of Gravenstein. Nebel (37) published a summary of his previous papers in 1930 and included with this a record of new chromosome counts which he had made. These new counts included 4

triploids, 4 diploids and one aneuploid (the last he questioned).

Thirty-one varieties and seventeen seedlings of cultivated apples were examined by Darlington and Moffett (7). They made both somatic and meiotic counts and confirmed Rybin (42) and Nebel's (37) statements that the haploid number in the apple was 17, and that apple varieties fall into two large groups, diploids with 2n = 34 and, triploids with 2n = 51.

Evreinoff (10) in 1931 found that the haploid number was not 17 for all varieties examined. Instead of 17 there were varieties with 12, others with 16, 18, 20, and 24 respectively. Further he maintained that Rybin was incorrect when he gave 17 as the haploid number, and that Shoemaker's (46) work on the variety, Delicious(Shoemaker gave this variety as 2n = 28) practically confirmed his theory that there existed in the apple various groups with constant chromosome numbers and that these differences could be used to explain the variation in external characters between apple varieties, and finally how the varieties themselves originated. Further, he endeavored to show that his series of chromosome numbers in the apple were analogous to those in Chrysanthemum and Crepis.

Among the latest publications dealing with chromosome counts in apples are those by Sax (44 and 45). He made chromosome counts on 15 species. In all cases the species

were either diploid, triploid or tetraploid. Aneuploids were not observed.

Moffett (32) in an excellent paper on the Pomoideae adds chromosome counts on three horticultural apple varieties. Also he examined the various groups of Pomoideae, all of which were orthoploid, having 34, 51, or 68 chromosomes. The diploid species were quite similar in that at the division of the pollen mother cell the separation of the chromosomes was usually perfect although sometimes there was a slight lagging of one univalent. Two types of triploids occurred, auto-triploids and allo-triploids. The latter were formed from a cross between a diploid and a tetraploid and had very irregular divisions with numerous univalents while the former usually formed only trivalents.

In an exhaustive treatise covering fruitfulness in the apple, pear, plum and cherry, Natividade (33) reported chromosome counts for 13 diploid and 6 triploid apple varieties. His data were in agreement with those reported by Nebel (37), Darlington and Moffett (7), Moffett (32), and Sax (44 and 45).

Further, Moffett's data (32) from diploid x triploid and triploid x triploid seedling studies supported Darlington and Moffett (7) and Crane and Lawrence's (4) assertion that crosses having one triploid parent produce aneuploid seedlings which are lacking in vigor.

In a study begun in 1932 and published in 1933 Kobel (38) gave chromosome counts on 103 seedlings which were produced through controlled crosses between 2n x 2n, 2n x 3n, 3n x 2n, and 3n x 3n varieties of apples. All the seedlings produced from crosses having at least one 3n parent were aneuploids. Only 16 out of 89 aneuploid seedlings were classified as having vigor, while all the seedlings from the diploid x diploid crosses were classified as vigorous.

An interesting report on the cytological behavior of 15 diploid and 2 triploid apple varieties in France was given by Miedzyrezecki (31) in 1933. He noted that diploid varieties were almost always regular and triploid varieties irregular in their reduction division. His germination studies on diploid and triploid varieties were in agreement with Kobel (18), Shoemaker (46), Heilborn (13 and 14) and Crane and Lawrence (4). Miedzyrezecki (31) also reported chromosome counts for three diploid Malus species.

In her study of cultivated varieties of apples, Roscoe (40) in 1934 reported fourteen diploids and four triploids. Aneuploids were not observed.

Heilborn (14) in a very complete report in 1935 reviewed reduction division, pollen lethality, and polyploidy in the apple. He presented a very complex pollen lethal theory, based upon Darlington and Moffett's "secondary polyploidy" (7) chromosome constitution in the apple as an explanation for the

pollen sterility which is found in diploid apple varieties.

This treatise and the previous literature review clarify several points which can be seen by examining tables 2 and 3 of the thesis. These points are namely:

(1) The chromosome constitution of the apple is, haploid = 17 and diploid, triploid (and one tetraploid) varieties are found. The aneuploids reported by Shoemaker (46), Kobel (15), Maney and Welter (30) and Heilborn (13) were not true aneuploids but were in reality either diploids or triploids in which the counts were based only on reduction division figures. Since the chromosomes in these figures are exceedingly small and often massed in groups it is very difficult to secure ortho-ploid counts. In fact, even in somatic plates this same condition is encountered, but to a less extent. A second reason for these aneuploid counts is perfectly evident. Most of these investigators did not have the best microscopic apparatus which would have assisted them to make more definite observations, (2) All of the investigators agree that the diploid varieties are practically always regular in their reduction division, (3) Again the literature shows that the triploids and the so-called "aneuploids" are irregular in their reduction division, (4) Data from Darlington and Moffett (7), Crane and Lawrence (4), Dahl (6), Moffett (32) and Nebel (38) indicate that crosses having one triploid parent produce aneuploid seedlings.

Table 2. LIST OF CHROMOSOME NUMBERS IN HORTICULTURAL APPLE VARIETIES.

	Natividade	Kobel	Rybin	Heilborn	Nebel	Darlington and Moffett	Crane and Lawrence	Moffett	Evreinoff	Roscoe	Mledzyrezecki
Adersleber Calville					34						
Åkerö				34							
Alexander				34							
Allington Pippin				34		34					
Annie Elizabeth						34					
Antonovka			34								
Antonovka Katmenitchka			34								
Apfel aus Lunow					34						
Aport			34								
Arkansas					34						
Astrachan big transp.				34							
Astrachan white			34	34					34		
Babuskino			34								
Baldwin	(48-49)				51	51					
Barlóvskoje			34								
Bauman's Reinette		36						34			
Beauty of Bath						34					
Belle de Boskoop		46			51						
Belle Fille											34
Belleflower yellow			34		34						
Belleflower x Kitaika of Mitchurin			34								
Belle Joséphine											34
Belvi Naliv			34								
Bemposta	51										
Ben Davis					34						
Berner Rosenapfel		34									
Blanche d'Espagne											34
Blenheim Orange						51	51				
Bohnapfel		49									
Borowinka-Borowitsky											34
Bramley's Seedling						51					

Table 2 (continued)

	Natividade	Kobel	Rybin	Heilborn	Nebel	Darlington	Land.Morfeat	Crane and Clarke	Moffett	Evrelnoff	Rosoe	Miedzyrzecki
Branco	34											
Bravo de Esmolfe	34											
Burro	34											
Calville blanc												34
Calville Lesans					34							34
Calville du roi			34									
Calville Grossheroz von Baden					34							
Calville blanche d'hiver									32			
Calville rouge									32			
Calville rouge d'automme									32			
Calville rouge d'hiver									32			34
Camoesa fina	34											
Canadian Reinette			51									
Candille Sinap			34									
Carlisle Pippin						34						
Carneira	51											
Casa Nova de Alcobaça	34											
Cellini		34		34								
Cerca	34											
Charlomowsky(Duchess of Oldenburg)				34	34							
Coimbra	34											
Cox's Orange-Pippin						34	34					
Cox's Orange-Reinette		34										
Cox's Pomona						34						
Crimson Beauty of New Brunswick										34		
Crimson Bramley						51	51					
Damason-Reinette		(45-47)										
Danzigeer Kantapfel		34										

Table 2 (continued)

	Natividade	Kobel	Rybin	Heilborn	Nebel	Darlington and Moffett	Crane and Lawrence	Moffett	Tyreinoff	Roscoe	Miedzyrzecki
Dash-Alma			34								
Deacon Jones										34	
Delicious (Shoemaker reported 2n = 28)										34	
Der Boëhmer					34						
Djir Hadzhi			34								
Dolgo					34						
Douchin (Malling Type II)						34					
Duchess Favorite						34					
Duchess										34	
Early Red Bird										34	
Early Victoria						34					
Eden					34						
Eneroth's Klaräpple				34							
Esopus Spitzenberg		34									
Espelho	51										
Fallawater										51	
Fenouillet jaune											34
Fenouillet rouge											34
Frösåker				51							
Géante des Expositions											34
Geheimerat Dr. Oldenburg					34						
Gelber Richard				34	34						
General von Hammerstein					34						
Genete Moyle						51					
Golden Reinette of Kursk			34								
Golden Russet										34	
Goldenreinette von Blenheim		40			51						
Grand Alexander								(32-34)			
Grågylling				34							
Gronho	34										
Gravenstein Banks Crimson										51	
Gravenstein		(45-46)		51	51						
Grenadier						34					

Table 2 (continued)

	Natividade	Kobel	Rybin	Heilborn	Nebel	Darlington and Moffett	Crane and Lawrence	Moffett	Evreinoff	Rosaqe	Miedzyrzecki
Grimes Golden					34					34	
Gule-Penbe			34								
Halland's Brunnsäpple				34							
Hampus				34							
Harbert's Reinette		45									
Hurlbut				51							
Husmodersäpple				51							
Irish Peach						34					
Jacques Lebel		(49-51)									
Jaune de Metz (Malling Type IX)						34	34				
Jonathan					34					34	
Kassaler-Reinette		34									
Kavlås				34							
Kentish Codlin						34					
Keswick Codlin						34					
Kola					68						
Lane's Prince Albert					51	34	34				
					34						
Lord Derby						34					
Lord Grosvenor			34								
Macoun					34						
Maglemer				34							
Manx Codlin						34					
Mank's Küchenapfel					34						
Margaretaäpple red				34							
McIntosh					34						
Medina					34						
Melápio	34										
Melba					34						
Melon				34							
Menznauer Jäpgerapfel (Rot Kanzler)		38									

Mercer County Seedling (Maney and Welter reported 2n = 26-30)

Table 2 (continued)

	Natividade	Kobel	Rybin	Heilborn	Nebel	Darlington and Moffett	Crane and Lawrence	Moffett	Evreinoff	Roscoe	Miedzyrzecki
Milton					34						
Minister von Hammerstein					34						
Muskat-Reinette		34									
110 Nomblot(Margille?)											51
Newfane					34						
Newton Wonder						34					
Nonpareil(Roxbury Russet)										34	
Nonsuch(Malling Type VI)						34					
Northern Spy						34	34				
Odlins						34					
Öland's Kungsäpple				34							
Old English Broadleaf Paradise(Malling Type I)						34					
Ontario					34						
Ontario Reinette		34									
Oranie				34							
Patte d'oie											34
Pearmain d'Adam											34
Pfirsichroter Sommerapfel		34									
Pipo	34										
P. J. Bergius				34							
Rambour de Himbsel											34
Rambour of Tasar. Koje Selo.				34							
Red Astrachan					34				34		
Red Siberian Crab					34						
Red Spy										34	
Reguenga	51										
Reine	34										
Reine des Reinettes									32		34
Reineta parda	51										
Reinette de Champagne		34,51[1]									
Reinette d'Oberdieck		34									
Reinette d'Orleano		34									
Reinette Bauman									34 36		

Table 2 (continued)

	Natividade	Kobel	Rybin	Heilborn	Nebel	Darlington and Moffett	Crane and Lawrence	Moffett	Eyreinoff	Rosgoe	Miedzyrzecki
Reinette Blenshiem									40		
Reinette du Canada	(38-40)										
Reinette grise d'automne									48		
Reinette grise d'hiver									48		
Reinette grise de Vitry											51
Reinette pain de sucre											34
Reinette Rouge d'Hiver										34	
Reinette très tardive											34
Reinette Zuccamaglio						34					
Rev. W. Wilks							34				
Reseda-Reinette	40										
Rhode Island Greening					51						
Ribston Pippin	42			51	51	51	51				
Rival						34					
Rome					34						
Rosenhäger				34							
Rosmarin blanc			34								
Rossvik				51							
Roter Eiserapfel	47										
Roter Jumpfernapfel					34						
Roter Stettiner			34								
Sary-Sinap			34								
Sary-Tursh-Alma			34								
Schöner von Boskoop	46				51						
Sävstaholm				34							
Signe Tillisch				34							
Skvoznoy Naliv			34								
Sommerrambour					34						
Sommergewurzapfel	34										
Sousa	34										
Spätblühender Taffetapfel					34						
Stäfner Rosenapfel	(48-49)										
Stäringe				34							
Stark										51	

Table 2 (continued)

	Natividade	Kobel	Rybin	Heilborn	Nebel	Darlington and Moffett	Crane and Lawrence	Moffett	Evreinoff	Roscoe	Miedzyrzecki
Stayman's Winesap (Shoemaker reported 2n = more than 28)											
Stenkyrka				34							
Suislepper			34								
Svanetrop				34							
Tchernoguz			34								
Tchulanovka			34								
Tâmara	34										
Titovka			34								
Tompkins King					51						
Transparente blanche				34							
Transparente de Croncels		34		34							
Transparente de Croncels x Weisser Astrachan		34									
Twenty ounce					34						
Vaughan Seedless					34						
Vitgylling				34							
Wagner										34	
Wealthy				34	34						
Warner's King		42									
Washington								34			
Weidner's Goldreinette					34						
Wellington										34	
Wellington Bloomless					34						
Winesap					34						
Winter Banana										34	
Winter Golden Pearmain		34,51[1]									
Winter Gray Reinette		34									
Winter Magetin						34					
Winter Zitronapfel	(48-49)										
Wolf River										34	
Worcester Pearmain						34					
Yellow Newton					34						
Yellow Transparent					34				34		
York Imperial										34	

Table 2 (continued)

	Natividade	Kobel	Rybin	Heilborn	Nebel	Darlingston and Moffett	Crane and Lawrence	Moffett	Evreinoff	Rosboe	Miedzyrzecoki
Zalenka Crimean		34									
Zwanzig Unzenapfel					34						

1
Refers to pages 31 and 33. Triploid as well as diploid seedlings were found. These varieties were found to show irregular divisions.

Table 3. LIST OF CHROMOSOME NUMBERS IN APPLE SPECIES.

Species[1]	Nebel	Sax	Rybin	Kobel	Darlington* and Moffett
M. adstringens		34			
". amurensis	34				
". angustifolia Michx.		66	34		
". baccata Borkh.	34	34	34		
". brevipes		34			
". coronaria Mill.	68	68			
". coronaria var. ioensis C. K. Schneid			65		
". Dawsoniana		34			
". eleyi(Miedzyrecki(31) reported the species 2n = 34)					
". floribunda Sieb.	34			34	34
". fusca Schneid	34				
". glaucescens Rehd.	68	68			
". Halliana Koehne	34			47	
". ioensis Britt.	54	(Maney and Welter (30)reported this species 2n = 28).			
". Malus Britt. (P. Malus L.)	34				34 and 51
". micromalus		34			
". Niedwetzkyana Dieck.	34			34	
". prunifolia Borkh. (?)	51±1	34	34		
". prunifolia macrocarpa	34				
". pumila var. paradisiaca C. K. Schneid("Paradise")			34	34	
". pumila var. praecox C. K. Schneid("Doucin")			34		
P. Ringo L.					34
M. rivularis	34				
". robusta		34			
". Sargenti Rehd.	34		(64-69)		
". Scheideckeri Zab.	34	34		34	
". Sieboldi Rehd.	34				
". Soulardi Britt.	34	34			
". spectabilis Borkh.	51[2]		34	(Miedzyrecki(31) Reported this species 2n = 34)	
M. sylvestris Mill.	34		34	34	

*Darlington and Moffett(7) report the apple under the generic name, Pyrus, the other authors (18)(30)(31)(34) and (41) under Malus.

Table 3 (continued)

Species[1]	Nebel	Sax	Rybin	Kobel	Darlington* and Moffett
M. theifera		51			
". Toringo Sieb.				(64-71)	
". Zumi Rehd.		34		34	

*The species have been tabulated as listed by the various authors except Darlington and Moffett's (7) counts. No attempt has been made to list the various synonyms or to group the synonyms together.

[1] Bailey (1) lists the following species of apples:
P. Malus, L. (Malus sylvestris, Mill., M. communis, D. C.).

P. Malus, var. paradisiaca, L. (Malus pumila, Mill.).

P. Malus, var. apetala, Aschers. and Graebn.

P. Malus, var. Niedwetzkyana, Aschers. and Graebn.(P. Niedwetzkyana, Hemsl.).

P. ioensis, Bailey(Malus ioensis, Britt.).

P. Soulardi, Bailey(Malus Soulardi,Britt.).

P. spectabilis, Ait.(Malus spectabilis,Borkh.).

P. prunifolia, Willd.(Malus prunifolia,Borkh.).

P. prunifolia, var. robusta,Bailey(Malus robusta,Rehd.).

P. prunifolia, var. Rinki, Bailey(Malus prunifolia,var. Rinki, Rehd., M. Ringo,Carr., Pyrus Ringo, Wenz.).

P. micromalus, Bailey(Malus micromalus, Makino., M. spectabilis, var. Kaido, Sieb., P. spectabilis, var. Kaido, Bean.).

P. angustifolia, Ait. (Malus angustifolia, Michx.).

1

P. coronaria, L. (Malus coronaria, Mill.).

P. Halliana, Voss. (Malus Halliana, Koehne).

P. Halliana, var. Parkmanii, Bailey.

P. baccata, L. (Malus baccata, Borkh.).

P. pulcherrima, Aschers. and Graebn. (P. floribunda, Hort., not Lindl., Malus floribunda, Sieb.).

P. pulcherrima, var. atrosanguinea, Bean(P. atrosanguinea, Spaeth., Malus atrosanguinea, Schneid.).

P. pulcherrima, var. Scheideckeri, Bailey(P. Scheideckeri, Spaeth., Malus Scheideckeri, Zabel).

P. pulcherrima, var. Arnoldiana, Bailey (Malus Arnoldiana, Sarg.).

P. Zumi, Mats. (Malus Zumi, Rehd.).

P. Sargenti, Bean(Malus Sargenti, Rehd.).

P. toringoides, Osborn (Malus toringoides, Hughes., P. transitoria, var. toringoides, Bailey).

P. Sieboldi, Regel(Malus Sieboldi, Rehd., Pyrus and Malus Toringo, Sieb.).

P. fusca, Raf. (Malus fusca, Schneid., Pyrus rivularis, Dougl.).

P. Dawsoniana, Bailey(Malus Dawsoniana, Rehd.).

2

First reported (34) 2n = 34. Later (35) as 51 but the last count was made on material which did not conform entirely to the described species.

EXPERIMENTAL

Materials

The varieties Hibernal, King David, Starking, Ames 541, Ames 550, Jonathan, Delicious, Grimes, Anisim, Stayman, Whitney, and Virginia Crab were examined cytologically. Flower buds were secured from trees in the experimental orchard at Ames, Iowa.

With the exception of the three varieties, Ames 541*, Virginia Crab and Ames 550, the above varieties are listed and described by Beach (2). Ames 541 is mentioned by Maney (30) as being a large fruited, open-pollinated seedling of the Mercer County Crab. This is the only published description of Ames 541. Virginia Crab according to Maney (25) is a definite variety discovered about 1885 at Muscatine, Iowa, among some Hewes Virginia Crab seedlings. He stated,"It is propagated by root grafting like any standard variety and is not a general line of miscellaneous seedlings as is sometimes conceived by those not familiar with the history of its origin." Ames 550 is a seedling produced by the Pomology Subsection, Iowa State College. It was recognized as being an excellent

*The parentage of Ames 541 and Ames 550 is: Ames 541 is an open-pollinated seedling of Mercer County Crab, and Ames 550 is a seedling from the cross, Briar Sweet x Mercer County Crab.

stock producer by Maney (27 and 28).

Methods

During the months, January, February and March, of 1932, apple branches were taken from the college orchard and were forced into bloom in the greenhouse. Later on, additional material was collected directly from the orchard. The flower buds were killed in Carnoy, Bouin, Allen's modification of Bouin, Nemec, Karpechenko, and other variations of the general chromo-acetic-formalin formula. Of these fluids, Carnoy and Bouin were found to be most satisfactory for chromosome counts, although in some instances the other fluids gave very good figures. Nemec was very useful for general structure. Karpechenko and Bouin were found to be best for somatic figures. Gentian-violet-iodine, safranin-fast-green, safranin-gentian-violet and iron haematoxylin were tested as stains. Since iron haemetoxylin was found to give the greatest differentiation between the cytoplasm and the chromosomes it was used throughout the investigation.

Unsuccessful attempts were made to secure somatic counts on Hibernal, Virginia Crab, Whitney, Ames 550 and the various Hibernal seedlings shown in figures 4 to 10, by forcing root cuttings in the greenhouse in the spring of 1933. The root tips from these cuttings were so small and weak that the

individual somatic cells were extremely small. These small, weak root tips contained somatic figures but these could not be counted accurately because the chromosomes were lying closely together.

However, large and vigorous root tips were obtained from open-pollinated seedlings of the various varieties which contained countable somatic divisions. These were secured merely by taking the seeds out of the fruit in February, 1933 and germinating them on moist blotting paper inside of a loosely covered petri dish. As soon as the radicle emerged, about one-eighth inch of the tip was removed for cytological study. This mutilation does not kill the seedlings because they flourished when transferred to moist sand.

Flower bud sections were cut from 8-10 microns in thickness and root tips from 4-6 microns. Some 3000 microscopic slides were made during the investigation. These were studied carefully with a low power microscope for mitotic or meiotic figures. When these were found, the material was examined with great detail using a Spencer binocular microscope equipped with a 90x oil-immersion objective and eyepieces having magnifications of 15, 20, and 30x.

Pollen morphological studies were made on binucleate, shed pollen grains in May, 1932. Similar pollen grains were germinated in petri dishes using a medium composed of 5% cane

sugar and 1% medium agar-agar and 94% distilled water by weight.

Drawings were made with a camera lucida using the combination of the 90x oil-immersion objective with the 15x, 20x or 30x oculars.

Cytological investigations

Whitney. This variety had regular reduction division. Occasionally one lagging chromosome was seen in the first division anaphase (plate I, figs. 1 and 2, and plate V, figs. 5 and 6). However, chromatin material was not observed outside the daughter nuclei following either the first or second division (plate I, figs. 3, 4, 5, 6, 7, and 15, and plate V, figs. 7, 9 and 10). The bivalent chromosomes in most of the metaphase plates were so close together that they could not be clearly distinguished and counted. However, one fairly clear metaphase plate of the first division was counted with 17 chromosomes (plate I, fig. 8). Thus the diploid chromosome number would be 34. In all instances, normal, four-celled tetrads were found (plate I, fig. 9). Microspores were found with nuclei containing one, two or three nucleoli (plate I, figs. 10, 11, and 12, and plate V, figs. 12 and 13). Uninucleate and binucleate pollen grains were observed in the anthers (plate V, figs. 14 and 15). Thus the first division of the pollen nucleus must take place inside the anther before the

pollen is shed. The generative nucleus was observed in the metaphase stage (plate I, figs. 18 and 19, and plate V, figs. 17 and 18). The division of the generative nucleus occurred after the pollen tube had grown considerably.

Pollen counts were made on dehisced pollen grains. 28.6 per cent of these were empty. One per cent germination was obtained with this variety.

Ames 550. This variety had regular reduction division. Figures 1, 3, 4, 5, 6, 13 and 20 of plate II illustrate this regularity. Seventeen bivalent chromosomes were counted at the first division metaphase (plate II, figs. 2 and 20). Therefore, the diploid chromosome number of the variety must be 34. Fifteen open-pollinated seedlings of this variety were examined somatically and each had a somatic count of 34 confirming the diploid count of 34 as obtained in the reduction division .

Figures 6, 7, 8, 9 and 13 of plate II illustrate the method of microspore formation in the apple. Instead of forming a permanent wall immediately after the first meiotic division, the cell wall is not formed until after the second meiotic division, when the four spores are delimited simultaneously by walls which are formed by furrows developing inward from the periphery. In the preparations, vacuoles did not seem to precede the furrowing. This observation should be

checked using other stains and killing fluids before being definitely accepted.

Normal tetrads were observed in all preparations (plate II, fig. 13). In a few cases, the microspores after being released from the pollen mother cell showed partial vacuolation. A high percentage of empty pollen grains was observed in all preparations (plate II, fig. 12 and 21).

Pollen counts were also made. Only 14.5 per cent empty grains were observed. Fourteen per cent germination was obtained.

Virginia Crab. This variety was very irregular in its reduction division (plate II, fig. 18, 19, 22, and 23, and plate V, fig. 1, 2 and 3). The pollen mother cells were normal in all respects. Chromosome counts ranging from 23 to 28 were made at diakinesis. Due to the smallness of the apple chromosomes and the irregularity in size of the clumps these counts cannot be relied upon. Anaphase counts were made and these also ranged from 23 to 29 chromosomes (plate III, fig. 8). Side views of first division anaphase partially explain these counts. In the side views the chromosomes are seen to divide at different times and in anaphase the number of lagging chromosomes is observed to be very high (plate II, fig. 18, and plate V, fig. 1). In fact, there are usually one or two masses of chromatin lying between the two poles at late anaphase and which are not included within the two daughter

nuclei but are seen in the cytoplasm during the telophase stage (plate V, fig. 2).

In the second division anaphase the number of lagging chromosomes was nearly as numerous as in the first division anaphase (plate II, fig. 23, and plate V, fig. 3). Practically every division showed from one to five lagging chromosomes. In the telophase of both the first and second division distinct masses of chromatin could be observed in the cytoplasm outside the daughter nuclei (plate II, figs. 19 and 22 and plate V, fig. 2).

Tetrad formation appeared to be normal in that 4-celled tetrads were formed (plate II, fig. 25). In some instances, vacuolated microspores were formed. This must have occurred between the time of tetrad formation and the final release of the microspores from the pollen mother cell wall.

Pollen counts were made on dehisced pollen grains. Forty per cent of these were found to be empty. Only one-fifth of one per cent germination was obtained with this variety.

Hibernal. Lagging chromosomes were observed in nearly all first division figures (plate III, figs. 1 and 2, and plate V, fig. 4). These lagging chromosomes failed to be included in the reorganized daughter nuclei (plate III, fig. 3). Figures of the second division anaphase were not available but this division also must show lagging chromosomes since second

division telophase figures show chromatin masses in the cytoplasm outside of the daughter nuclei (plate III, figs. 5 and 6). Counts were made of first division metaphase figures but these were variable in number, since bi, tri, and other multivalent associations were common. Figure 4 of plate III shows a first metaphase with 24 chromosome bodies, three of which have formed a sexivalent. Again in fig. 7, plate III, a first division anaphase figure is shown with 24 chromosomes at one end and 24 or 25 chromosomes in the other. There are three small chromatin masses in this plate, one in the lower figure and two in the upper figure. These appear to be very small chromosomes. If this is true, the chromosome count of this variety must be 51 or 52.

The tetrads were very highly vacuolated in some preparations while in others they were apparently normal (plate III, figs. 9 and 14). In rare cases bodies which took the chromatin stain were distinguishable in the early uninucleate stage of the microspore (plate III, fig. 12).

Approximately 37 per cent of the shed pollen was empty and only one-tenth of one per cent germination was obtained.

Anisim. This variety was regular in its reduction division. Seventeen chromosomes were counted at second metaphase in one figure and two sets of 17 univalent chromosomes were counted in a first division anaphase figure (plate IV, figs. 1 and 2). Therefore, this variety is to be accepted as

a diploid with 34 chromosomes.

Starking. Both first and second division metaphase figures showed 17 chromosomes (plate IV, figs. 3 and 6). Sometimes the second division metaphase figures were difficult to count, since there was a premature separation of the chromosome halves for the following anaphase division (plate IV, fig. 3).

Two seedlings of Starking were examined somatically and in each case a diploid count of 34 was obtained.

Delicious. The entire reduction division was regular. This variety showed mostly 16 bivalent chromosomes in the first division metaphase figures. With difficulty some of the metaphase plates can be broken up into 17 pairs. The preceeding variety, Starking, a bud sport of Delicious, is a diploid with 34 chromosomes. Bud sports have been found in Gravenstein to have the same chromosome constitution as the parent variety Nebel (37). It is generally recognized that Starking has darker red color than Delicious, colors earlier in the fall, bears earlier, and has a somewhat stronger tree structure. All of these characteristics point to a chromosomal change analogous to those experienced in Drosophila where one mutation often affects more than one external characteristic. Hence, it seems that while only 16 bivalent chromosomes can be distinguished in the majority of the first metaphase figures, the

true count is 17 bivalent chromosomes. Still further evidence was secured from four open-pollinated seedlings of Delicious which had a somatic count of 34 chromosomes. This conclusion was later confirmed by Roscoe (40) who has reported Delicious as a diploid with 34 chromosomes.

Shoemaker (46) no doubt determined the count as 28 because he did not study somatic figures and took clumps of several chromosomes in the metaphase figures to represent one chromosome pair.

King David. King David is a diploid with 17 chromosomes in the haploid condition. Second division metaphase counts were made showing 17 bivalent chromosomes in each figure(plate IV, fig. 7). The reduction division processes were regular.

Jonathan. All reduction division processes were regular in this variety. A second division anaphase plate was observed which showed four sets of 17 chromosomes (plate IV, fig. 9). Therefore, Jonathan is a diploid with 34 chromosomes.

Stayman. This variety was irregular in its reduction division. Lagging chromosomes were observed in practically every first division anaphase figure. Masses of chromatin were observed in the cytoplasm at the second division prophase. In one clear first division anaphase figure, 20 chromosomes were observed in one nucleus and 27 in the other (plate IV, fig. 4). In a clear second division metaphase plate one

figure showed a count of 24; the other figure was uncountable (plate IV, fig. 5). Therefore, the somatic count for Stayman must be at least 47. This is considerably higher than Shoemaker's (46) report of more than 28 chromosomes. Eleven open-pollinated seedlings of Stayman were examined somatically. An exact chromosome count could not be made on all these seedlings because the chromosomes lay so close together but approximate counts were made. The first seedling had 44 or 48, the second, 40, the third 38 or 40, the fourth 36 or 38, the fifth 42, the sixth 40, the seventh 38, the eighth 40, the ninth 40, the tenth 46 and the eleventh 39. The irregular condition as seen in pollen mother cell figures and the aneuploid nature of the seedlings seem to justify the conclusion that Stayman is a triploid with 51 chromosomes.

Ames 541. This variety was irregular in its reduction division. This is in agreement with the observations of Maney and Welter (30). Another interesting fact is observed in the preparations. Maney and Welter (30) reported the presence of micronuclei in this variety. The preparations observed confirmed their findings. In the other varieties, wherever chromatin was seen in the cytoplasm, it seemed to be only in individual chromosomes, but in this variety there seemed to be an accumulation of several chromosomes in each clump of chromatin (that is, the size of the clumps was larger than one

chromosome). Accurate counts on this variety have not been made.

This variety must be triploid since standard varieties with an aneuploid chromosome constitution do not exist in the apple (Darlington and Moffett (7).

Grimes. Diakinesis figures with 17 chromosomes were abundant. Also, second division metaphase plates occurred with 17 bivalent chromosomes (plate IV, fig. 8). Hence, it is concluded that Grimes has a diploid chromosome number of 34.

Chromosomal irregularities such as lagging were not seen in any of the preparations. Only normal tetrads were observed.

Table 4 shows the relative amount of lagging in anaphase figures of the first division in different apple varieties. It illustrates how the triploid varieties, Hibernal, Virginia Crab and Stayman, show lagging chromosomes in practically all spindles, while the diploid varieties, Ames 550, Whitney, King David, Jonathan, Pyrus Baccata, Starking, Delicious and Salome show scarcely any figures with lagging chromosomes.

Table 4. COMPARISON OF OBSERVED NORMAL AND "ABNORMAL" FIRST DIVISION ANAPHASE FIGURES IN DIPLOID AND TRIPLOID VARIETIES.

Variety	Number of anaphase figures		
	Total	"Normal"	"Abnormal"
Hibernal (3n)	49	1	48
Stayman (3n)	46	1	45
Virginia Crab (3n)	90	2	88
Ames 550 (2n)	15	12	3
Whitney (2n)	50	43	7
King David (2n)	24	23	1
Jonathan (2n)	51	49	2
Pyrus baccata (2n)	60	60	
Starking (2n)	100	100	
Delicious (2n)	51	51	
Grimes (2n)	20	20	
Salome (2n)[1]	50	50	

[1] Its chromosome behavior would indicate that it was a diploid.

Again in table 5 the diploid varieties are shown to be almost lacking in lagging chromosomes in the 2nd division, while the triploid variety, Virginia Crab, shows lagging chromosomes in practically every spindle.

Table 5. COMPARISON OF OBSERVED NORMAL AND "ABNORMAL" SECOND DIVISION ANAPHASE FIGURES IN DIPLOID AND TRIPLOID VARIETIES.

	Number of 2nd division anaphase figures		
	Total	"Normal"	"Abnormal"
Ames 550 (2n)	100	100	0
Whitney (2n)	100	100	0
Jonathan (2n)	100	97	3
King David (2n)	23	21	2
Virginia Crab (3n)	100	4	96
Salome (2n)[1]	8	8	0

[1] Salome's chromosome behavior would indicate that it was a diploid.

From these tables it is evident that the triploid condition in the varieties, Hibernal, Virginia Crab, and Stayman is invariably associated with irregular division and lagging chromosomes, while the diploid varieties, Ames 550, Whitney, Jonathan, King David, Salome, Pyrus baccata, Grimes, Starking and Delicious are regular in their reduction division.

Table 6. COMPARISON OF THE NUMBER AND PERCENTAGE OF EMPTY AND "NORMAL" OR FULL POLLEN GRAINS WITHIN THE ANTHERS OF AMES 550, WHITNEY, HIBERNAL AND VIRGINIA CRAB.

Variety	Pollen grains		
	Total Number observed	Empty Per cent	"Normal" or full Per cent
Ames 550 (2n)	214	14.5	85.5
Whitney (2n)	301	28.6	71.4
Hibernal (3n)	173	37.1	63.2
Virginia Crab (3n)	74	40.5	59.5

Table 6 shows that all four varieties possess a large percentage of empty pollen grains but that the triploid varieties have a much higher percentage than the diploid varieties.

Fruit setting in reciprocal crosses involving the triploid varieties, Hibernal and Virginia Crab

Tables 7 and 8 show the results of crosses involving four varieties, Hibernal, Virginia Crab, Ames 550, and Whitney, made during the years 1931 and 1932.

Very few crosses were successful in the spring of 1931 because of a killing frost at pollination time. Over 4200 flowers were emasculated and cross-pollinated from which only four Whitney fruits were secured, which bore twenty-six seeds. These were germinated in the spring of 1932, sixteen being viable. Fifteen of these seedlings were alive on October 15, 1932. Table 7 shows the data on these four fruits and their seeds.

Table 7. FRUIT SETTING IN CROSSES BETWEEN WHITNEY ♀, AND AMES 550 ♂, DUCHESS ♂, GREEN SWEET ♂ IN 1931 SHOWING NUMBER OF FLOWERS CROSS-POLLINATED, NUMBER OF SEEDS SECURED AND GERMINATING AND NUMBER OF SEEDLINGS ALIVE ON OCTOBER 15, 1932.

Breeding Number	Parentage[1]	Flowers pollinated	Seeds obtained	Seedlings obtained	No. of seedlings alive Oct. 15, 1932*
31701	Whitney x Ames 550 (2n x 2n)	260	16	10	9
31703	Whitney x Duchess (2n x 2n)	418	7	4	4
31704	Whitney x Green Sweet (2n x 2n?)	193	3	2	2

*All seedlings alive on October 15, 1932 possessed a high degree of vigor.

[1]The ♀ parent is written first in each cross.

Table 8 presents the results in seed production and fruit setting of the cross-breeding carried on in the spring of 1932. This table shows that; (1) Virginia Crab set only 4 fruits out of 463 cross-pollinated blossoms; (2) Hibernal set no fruits although 1,141 blossoms were cross-pollinated; (3) Ames 550 set 9 fruits from 1,323 cross-pollinated blossoms; (4) Whitney set 387 fruits out of 1,401 blossoms; (5) reciprocal crosses between Hibernal and Virginia Crab were complete failures; (6) where large enough numbers are available it is seen that diploid x triploid crosses are decidedly more unsuccessful than diploid x diploid crosses.

Table 8. FRUIT SETTING IN CROSSES INVOLVING SOME DIPLOID AND TRIPLOID VARIETIES IN 1932, SHOWING NUMBER OF FLOWERS POLLINATED, NUMBER AND PERCENTAGE OF FLOWERS SETTING FRUIT, TOTAL NUMBER OF SEEDS SECURED AND AVERAGE NUMBER OF SEEDS PER FRUIT.

Breeding number	Parentage*	Number blossoms pollinated	Number fruit set	Per cent pollinated flowers setting fruit	Total number seeds	Average number seeds per fruit
32010	Whitney x Ames 550 (3n x 2n)	397	170	42.8	1,253	7.4
32011	Whitney x Virginia Crab (2n x 3n)	240	4	1.7	9	2.2
32012	Whitney x Hibernal (2n x 3n)	487	33	6.6	124	3.8
32013	Whitney x Duchess (2n x 2n)	277	180	64.6	1,292	7.2
32015	Ames 550 x Whitney (2n x 2n)	425	6	1.4	32	5.4
32016	Ames 550 x Virginia Crab (2n x 3n)	430	3	0.7	22	7.3
32017	Ames 550 x Hibernal (2n x 3n)	470	0	0.0		
32018	Virginia Crab x Whitney (3n x 2n)	192	4	2.1	12	3.0
32019	Virginia Crab x Hibernal (3n x 3n)	166	0	0.0		
32020	Virginia Crab x Ames 550 (3n x 2n)	105	0	0.0		

Table 8 (continued)

Breeding number	Parentage*	Number blossoms pollinated	Number fruit set	Per cent pollinated flowers setting fruit	Total number seeds	Average number seeds per fruit
32021	Hibernal x Whitney (3n x 2n)	458	0	0.0		
32022	Hibernal x Ames 550 (3n x 2n)	351	0	0.0		
32023	Hibernal x Virginia Crab (3n x 3n)	352	0	0.0		
	Totals	4,350	422		2,885	

*The female parent is written first in each cross.

In 1932 cross-pollination of Ames 550 were mostly failures. This variety blooms much earlier in the spring than the other varieties used in the fruit breeding work. In 1932 the weather conditions during the Ames 550 blossoming period were unfavorable for pollination. Temperatures were low and scarcely a day passed without some precipitation. These conditions explain why this variety set so few fruits in 1932 when used in crosses involving diploid varieties.

As the weather conditions were favorable, when the other three varieties were pollinated, it seems fair to conclude that some factor other than weather conditions was responsible for the poor results whenever Virginia Crab or Hibernal was used in the crossing work.

Table 9 shows a comparison of the three varieties, Virginia Crab, Hibernal and Ames 550, in respect to natural set in 1932. From this table it is self-evident that the diploid, Ames 550, has a much higher natural set than either of the triploids, Virginia Crab or Hibernal. It is also evident that Ames 550 sets a higher percentage of its blossoms than the standard diploid varieties where usually 3 to 5% will produce a normal crop. More than likely this characteristic is inherited from Pyrus baccata. It is true that there may be physiological as well as chromosomal factors involved in the fruit setting of these varieties. Hibernal is a large apple,

while both Virginia Crab and Ames 550 have small fruit. However, even if due allowance is made for these factors, the differences are so large that it seems that the chromosomal factor may be the main one involved.

Table 9. COMPARISON OF THE APPLE VARIETIES VIRGINIA CRAB, HIBERNAL AND AMES 550 IN RESPECT TO NATURAL SET OF FRUIT IN 1932.

Variety	Number of blossoms	Number of fruit set	Percentage of blossoms
Hibernal	360	8	2.2
Virginia Crab	300	17	5.7
Ames 550	165	100	60.6

DISCUSSION AND CONCLUSIONS

Chromosome constitution of diploids

The preceeding data have shown that the varieties, Ames 550, Whitney, Jonathan, King David, Starking, Delicious, Anisim, and Grimes are diploids having 34 chromosomes. In practically all instances the reduction division is regular although in a few divisions of Whitney a slight lagging of one univalent was observed after the other chromosomes had reached the poles.

Chromosome constitution of triploids

The chromosome constitution of Hibernal, Virginia Crab, Stayman and Ames 541 was not so definitely determined by counts of meiotic figures. The large number and small size of the apple chromosomes interferred with counting in spite of good preparations and high magnification. First division anaphase figures showed 23 to 29 chromosomes in Virginia Crab, 24 and 25 in Hibernal, and 20 and 27 in Stayman. There were no counts of Ames 541.

In spite of failure to obtain full triploid counts of 51 chromosomes in somatic cells or first division anaphase counts of 25 and 26, the author is convinced that these varities are true triploids and not aneuploids. These convictions are based on the following facts:

1. All four varieties, had irregular reduction division.

2. Hibernal and Virginia Crab had a high percentage of empty pollen grains and the apparently normal pollen germinated very poorly.

3. As parents in fruit setting studies Hibernal and Virginia Crab proved to be unproductive as shown in Table 8.

4. Eleven open-pollinated seedlings of Stayman were aneuploids and the seeds obtained from Hibernal and Virginia Crab crosses failed to germinate. Also the Hibernal and Virginia Crab seedlings observed by Maney (26, 27 and 28) were lacking in vigor. (Figs. 1-3). Finally, Hibernal as a parent produced only a few weak trees . (Table 1 and figs. 4-10). (Lantz 20).

5. These varieties are vigorous growing trees while all the aneuploid seedlings observed as well as those reported by Darlington and Moffett (7) Moffett (32) and Nebel (38) are weak growing.

6. Finally, where aneuploid varieties have been reported from meiotic material only, Kobel (18),Heilborn (13), and Shoemaker (46), and have been re-examined using both somatic and meiotic material these so reported aneuploids have been found to be triploids. (Darlington and Moffett(7), Nebel (36) and Roscoe (40).

Pollen studies of diploid and triploid varieties

The data showed that the triploid varieties had a greater percentage of defective pollen than the diploids. Chromosomal irregularities in triploid varieties may explain the

relatively greater ineffectiveness of their pollen, but since diploid varieties also have considerable defective pollen, all defectiveness cannot be attributed to chromosomal irregularity. With diploid varieties practically regular reduction division was observed with normal tetrad formation.

Two other explanations have been advanced as possible causes for defective pollen (1) environmental conditions during pollination and (2) lethal factors. Heilborn (13) and Miedzyrzecki (31) observed that pollen sterility in the apple increased when temperatures were higher than normal. Heilborn concluded that normal temperatures in Sweden were too low to be a factor in diploid sterility. Hence, Heilborn (13 and 14) advanced the theory of pollen lethals to account for diploid sterility. Heilborn's theory seems logical because instances of pollen lethality have been reported by Sansome and Philp (45). Heilborn uses Darlington and Moffett's (7) theory of "secondary polyploidy" which postulates only seven original chromosomes, 3 of which are (chromosomes A, B, and C) triplicated and four of which (chromosomes D, E, F, and G) are doubled to comprise the haploid chromosome set in the apple. Heilborn further supposes that there is one system of pollen lethals situated in the group of A-chromosomes and another set of such lethals in the D-chromosomes. Pollen grains having either of the pollen lethal systems in the homozygous recessive condition shrivel and die.

It is generally accepted that the present day apple varieties are hybrids developed from inter-specific crosses. Hence, it might follow that they are characterized by chromosome constitutions possessing certain varying types of pollen lethal systems, and when varieties are crossed these various lethal systems result in different levels of sterility.

The pollen formation studies resolved themselves into an inquiry into the causes of sterility, for which the author offers the following explanations:

1. First, unfavorable environmental conditions can and do reduce fertility, possibly in a similar degree for all varieties, except those whose inherent chromosome constitution makes them especially susceptible.
2. Second, there appears to be a set or sets of pollen lethal systems which produce pollen sterility in the apple when certain gene combinations are encountered in the haploid pollen grain whether in diploid or triploid varieties.
3. And, finally, in triploid varieties, the unbalanced aneuploid chromosome number of the haploid pollen grain produces an additional and dominating explanation for the high degree of sterility in such varieties.

Fruit setting in reciprocal crosses involving the triploid varieties, Hibernal and Virginia Crab

The cross-breeding data shown in Table 8, agrees with that observed by Lantz (20) in that the triploid, Hibernal,

is an extremely poor breeding parent. It seems without a doubt that the conflicting evidence advanced by Beaumont (3) and the experimental farm at Lennoxville, Quebec (23) as to Hibernal's behavior was due to their having a strain of Russian apple which had been named Hibernal but which was cytologically distinct from that strain used in this investigation. The lack of germination of the Hibernal and Virginia Crab seeds confirms the findings of Lantz (20) and Maney (26 and 27).

In future cross-breeding programs involving triploids, little time should be spent on 2n x 3n crosses since triploid pollen is largely ineffective. Generally speaking triploids should not be included in a practical breeding program since they are very likely to yield a dearth of results.

General

The behavior of the Hibernal and Virginia Crab seedlings, which are lacking in vigor, is explained by the thesis observations which show that,

1. Hibernal, Virginia Crab, Ames 541, and Stayman have irregular reduction division,
2. The pollen of Hibernal, and Virginia Crab is low in fertility and germination,
3. Hibernal and Virginia Crab are unproductive when used in fruit setting studies (Table 8),

4. Hibernal and Virginia Crab seeds germinate very poorly (Maney 25, 26, and 27), and (Lantz 20),

5. The eleven open-pollinated seedlings of Stayman were aneuploids.

These triploid varieties through their irregular reduction division produce microspores so lacking in vigor due to their unbalanced chromosome constitution that few of them ever reach the pollen grain stage in a viable condition. Very few that progress to this stage are likely to form sperms and fertilize egg cells. Thus the effects of the unbalanced chromosome number are encountered in all stages of reproduction and growth of the seedlings with a consequent mortality in the triploid seedling population at all stages.

Diploid varieties, such as Whitney and Ames 550, on the other hand, in spite of the fact that they show some pollen sterility and sometimes faulty seed development, regularly produce far more vigorous seedlings than triploids. This ability to produce vigorous seedlings is associated in the diploid varieties with such behavior as the data have shown, namely, (1) regular reduction division, (2) relatively low percentage of pollen sterility, (3) higher percentage of pollen germination than triploid varieties, (4) high degree of fertility as male or female parents in fruit setting studies, (5) good seed germination, and, (6) orthoploid chromosome con-

stitution of open-pollinated seedlings from diploid varieties.

While seedlings of crosses between diploids are more vigorous than seedlings of triploids, variations in vigor among diploids themselves must be recognized. Evidence has been shown by Lantz and his co-workers (9, 19, 21, and 22), and Edgecombe (8) that seedling progenies from diploids differ in their mean level of vigor.

It is suggested that cytological studies dealing with the evolution of the apple might well take the form of studies of seedlings from reciprocal crosses between 3n x 2n, and 2n x 4n forms, using horticultural varieties and Malus species in an attempt to ascertain whether triploid forms could be produced. The cultivated variety, Kola, which is 4n should be used with 2n and 3n parents in an effort to produce 3n types of horticultural value.

However, since relatively barren results are secured from 3n cross-breeding programs, it would be well to supplement the above with cytological studies of open-pollinated seedlings from 3n varieties. To date no one has reported the pollen mother cell development of an aneuploid seedling from a 3n parent due to the usual cause -- death of the aneuploid before fruiting age. This should be investigated for information on reduction division in aneuploid seedlings.

The possibility of utilizing triploids in a different way from any thus far reported which might yield surprising results, namely, the securing of somatic doubling of the chromosome number in the 2n, 3n, and 4n forms is also suggested. Crane and Lawrence (5) point out that higher members of a polyploid series usually present a greater range of selection along horticultural lines. Hence, if such doubling could be secured the now useless 3n breeding varieties might well become excellent 6n varieties of extremely high value in themselves. The asexual propagation methods used with the apple would make perpetuation of such varieties a very simple and practical matter.

SUMMARY

1. A cytological study was made of twelve apple varieties and their seedlings in an effort to explain why, Hibernal and Virginia Crab produce seedlings lacking in vigor, while Ames 550 and Whitney produce vigorous seedlings.

2. The diploid varieties, Whitney, Ames 550, Anisim, Starking, Delicious, King David, Jonathan, and Grimes, were found to be 2n= 34, to have regular reduction division, high percentage of normal pollen, a relatively high germination of pollen, and when used in crossing with diploids to be productive.

3. The triploid varieties, Hibernal, Virginia Crab, Stayman and Ames 541, were found to be 2n as follows, Hibernal (at least 49), Virginia Crab (at least 46), and Stayman (at least 47). No counts were made on Ames 541. Since these counts were made from meiotic divisions only and since vigorous growing aneuploids are unknown these varieties are assumed to be triploids with 51 chromosomes. They were found to have irregular reduction division, poor pollen germination, a relatively low percentage of normal pollen, and when used in crossing with either diploids or triploids the crosses were usually complete failures.

4. All open-pollinated seedlings from diploid varieties when examined cytologically gave a chromosome count of 2n = 34 and were vigorous in growth.

5. All cytological examinations on open-pollinated seedlings from triploid varieties showed these seedlings to be aneuploids with a somatic chromosome constitution between 34 and 51.

6. Hence, the lack of vigor of Hibernal and Virginia Crab seedlings is explained on the basis of their irregular chromosome behavior during meiosis, gametes being formed with an unbalanced chromosome constitution, which on union with the female gametes produce aneuploid individuals, these seedling individuals being constitutionally lacking in vigor due to their abnormal chromosome constitution.

7. The varieties, Whitney and Ames 550, produce more vigorous seedlings than the triploids, Hibernal and Virginia Crab, because their reduction division processes are normal and consequently the gametes and finally, the seedling offspring when produced from crosses with other diploids are orthoploid.

8. Finally, the data substantiate the work of Darlington and Moffett (7), Crane and Lawrence (4), Moffett (32), Nebel (38), Heilborn (14), and Dahl (6) in that, chromosome number in apple seedlings is associated with vigor in triploid crossed where aneuploidy is a factor.

EXPLANATION OF PLATE I
(Camera lucida drawings)

Figures 1 to 19 inclusive of Whitney. 2000x except figure 19 which is 1200x.

Fig. 1. Side view, first late anaphase.

Fig. 2. Side view, first late anaphase with one lagging chromosome.

Figs. 3-4. First telophase figures; in fig. 3 the nucleoli are almost equal in size, in fig. 4 one member of each pair is decidedly larger than the other.

Fig. 5. Regular second anaphase.

Fig. 6. Early second telophase; small nucleoli and many masses of fused chromatin.

Fig. 7. Variable number of nucleoli at late telophase.

Fig. 8. Polar view, first metaphase plate showing 17 bivalent chromosomes.

Fig. 9. Normal tetrad.

Figs. 10, 11 and 12. Normal microspores with one, two and three nucleoli respectively. (Within the anther).

Fig. 13. Binucleate pollen grain; vacuolation in the cytoplasm.

Fig. 14. Side view, second metaphase plate.

Fig. 15. Side view, early second anaphase.

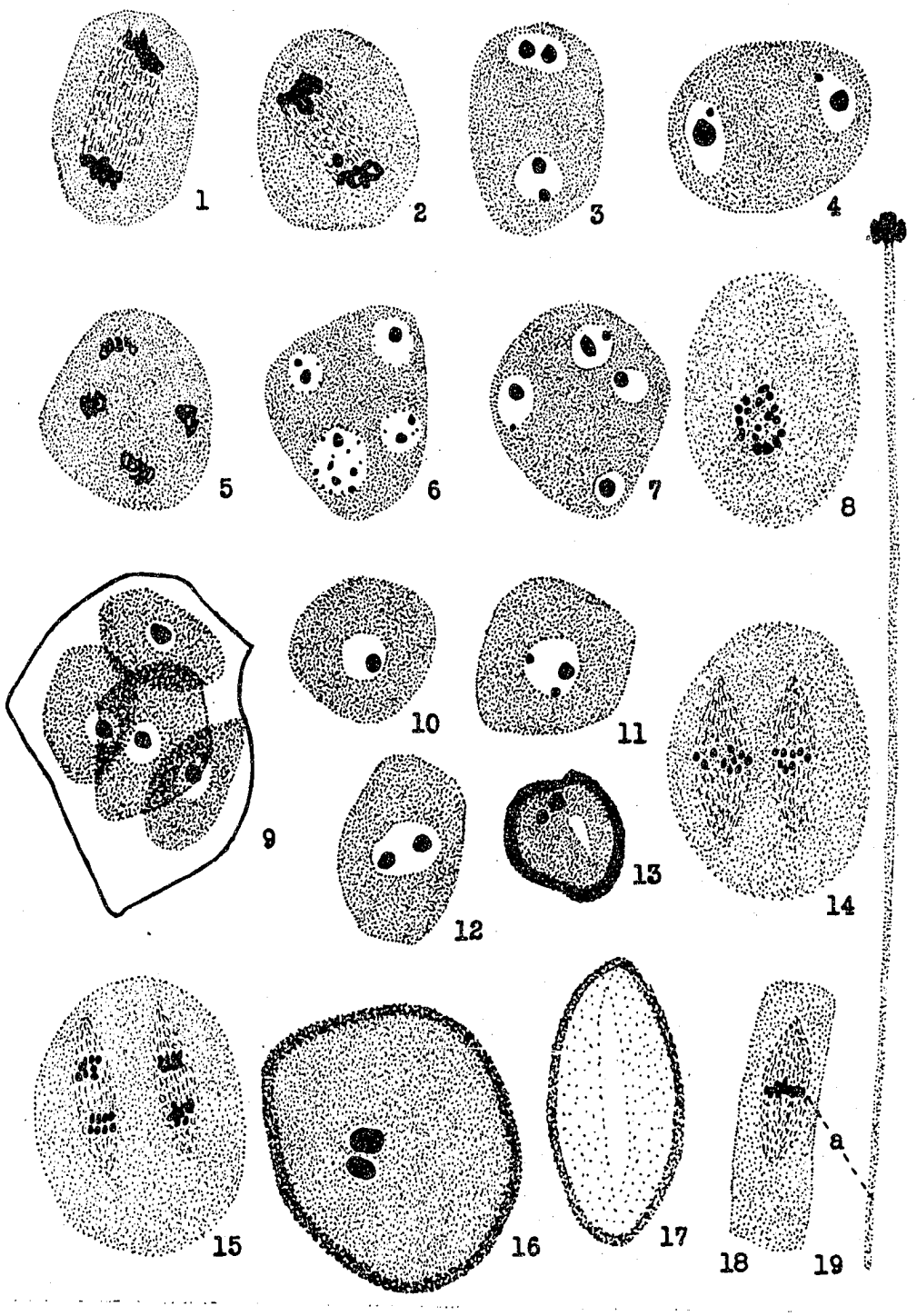
Fig. 16. Binucleate pollen grain.

Fig. 17. Empty pollen grain.

Fig. 18. Section of pollen tube containing side view of metaphase plate of generative nucleus.

Fig. 19. "a" shows location in the pollen tube of metaphase plate of fig. 18, about 150x.

PLATE I



EXPLANATION OF PLATE II
(Camera lucida drawings, 2000x).

Figures 1 to 17 inclusive and 20 and 21 are of Ames 550. Figures 18 and 19, and 22 to 25 inclusive are of Virginia Crab.

Fig. 1. Side view first anaphase.

Fig. 2. First metaphase plate with 17 bivalents and one fragment.

Fig. 3. First telophase.

Fig. 4. Side view second metaphase.

Fig. 5. Side view early second anaphase.

Fig. 6. Late telophase second division.

Figs. 7, 8, 9, 13. Formation of tetrads; fig. 7, pinching in process of outside walls; fig. 8, three members of tetrad just separated; fig. 9, tetrad showing two microspores slightly separated; fig. 13, tetrads completely separated.

Fig. 10. Normal microspore; two nucleoli present.

Fig. 11. Normal microspore; one nucleolus present.

Fig. 12. Empty microspore.

Fig. 14. Microspore released from pollen mother cell wall; vacuolation evident; cell wall has thickened.

Fig. 15. Binucleate pollen grain; note thickened cell wall.

Fig. 16. Uninucleate pollen grain.

Fig. 17. Dehisced binucleate pollen grain; cell wall much thickened.

Fig. 18. Side view first anaphase; four lagging masses of chromatin.

Fig. 19. Second prophase; four micro-nuclei in the cytoplasm .

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Fig. 15. Binucleate pollen grain; note thickened cell wall.

Fig. 16. Uninucleate pollen grain.

Fig. 17. Dehisced binucleate pollen grain; cell wall much thickened.

Fig. 18. Side view first anaphase; four lagging masses of chromatin.

Fig. 19. Second prophase; four micro-nuclei in the cytoplasm .

Fig. 20. First anaphase plate with two sets of 17 univalent chromosomes.

Fig. 21. Empty dehisced pollen grain.

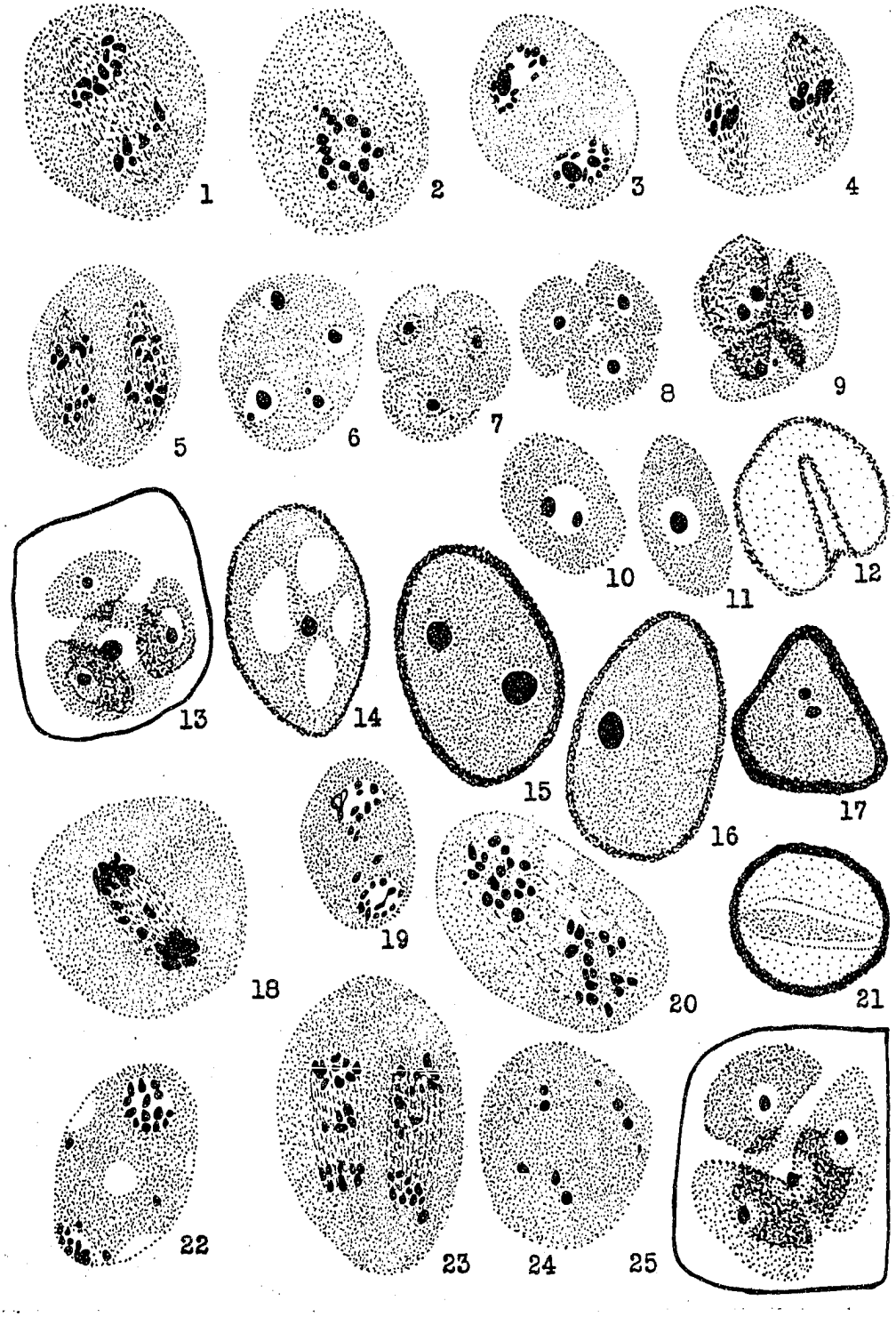
Fig. 22. Second telophase; four chromatin masses in the cytoplasm; vacuolation present.

Fig. 23. Side view second anaphase; lagging chromatin masses in both spindles.

Fig. 24. Second telophase; four nuclei present. Note lack of stained membranes.

Fig. 25. Normal tetrad formation.

PLATE II



EXPLANATION OF PLATE III
(Camera lucida drawings)

Figure 8, Virginia Crab, 2000x; the remainder of the plate of Hibernal; figures 13, 15, 16, 1200x, the other figures 2000x.

Fig. 1. First anaphase with five masses of lagging chromatin; one mass of chromatin at upper left hand of cell.

Fig. 2. Similar first anaphase with two lagging trivalents (?) and one lagging bivalent.

Fig. 3. Early first telophase showing five masses of chromatin in the cytoplasm. The nucleoli are distinct and take a very heavy nuclear stain.

Fig. 4. Polar view first metaphase showing 21 bivalents, and 1 sexivalent.

Fig. 5. Late second telophase showing 3 masses of chromatin in the cytoplasm; one or two nucleoli in the nucleus. The dotted lines indicate the "pinching in" process which precedes tetrad formation.

Fig. 6. An extreme case of abnormality in second telophase. 3 nuclei are formed; the fourth has failed to form a nuclear membrane and the chromatin is released into the surrounding cytoplasm.

Fig. 7. Side view first anaphase showing 24 chromosomes in one plate and 25 in the other. Note "a" this chromosome may be uni- or bi-valent. There are three very small staining bodies in the cell which might be chromosomes. If so this cell would show the triploid count of 51 chromosomes.

Fig. 8. Virginia Crab: polar view first anaphase with 29

Fig. 5. Late second telophase showing 3 masses of chromatin in the cytoplasm; one or two nucleoli in the nucleus. The dotted lines indicate the "pinching in" process which precedes tetrad formation.

Fig. 6. An extreme case of abnormality in second telophase. 3 nuclei are formed; the fourth has failed to form a nuclear membrane and the chromatin is released into the surrounding cytoplasm.

Fig. 7. Side view first anaphase showing 24 chromosomes in one plate and 25 in the other. Note "a" this chromosome may be uni- or bi-valent. There are three very small staining bodies in the cell which might be chromosomes. If so this cell would show the triploid count of 51 chromosomes.

Fig. 8. Virginia Crab; polar view first anaphase with 29 chromosomes.

Fig. 9. Tetrads with dense cytoplasm.

Fig. 10. Vacuolated microspore released from pollen mother cell wall.

Fig. 11. Chromosome grouping in first metaphase and anaphase plates.

Fig. 12. Microspore with dense cytoplasm and distinct masses of chromatin in the cytoplasm.

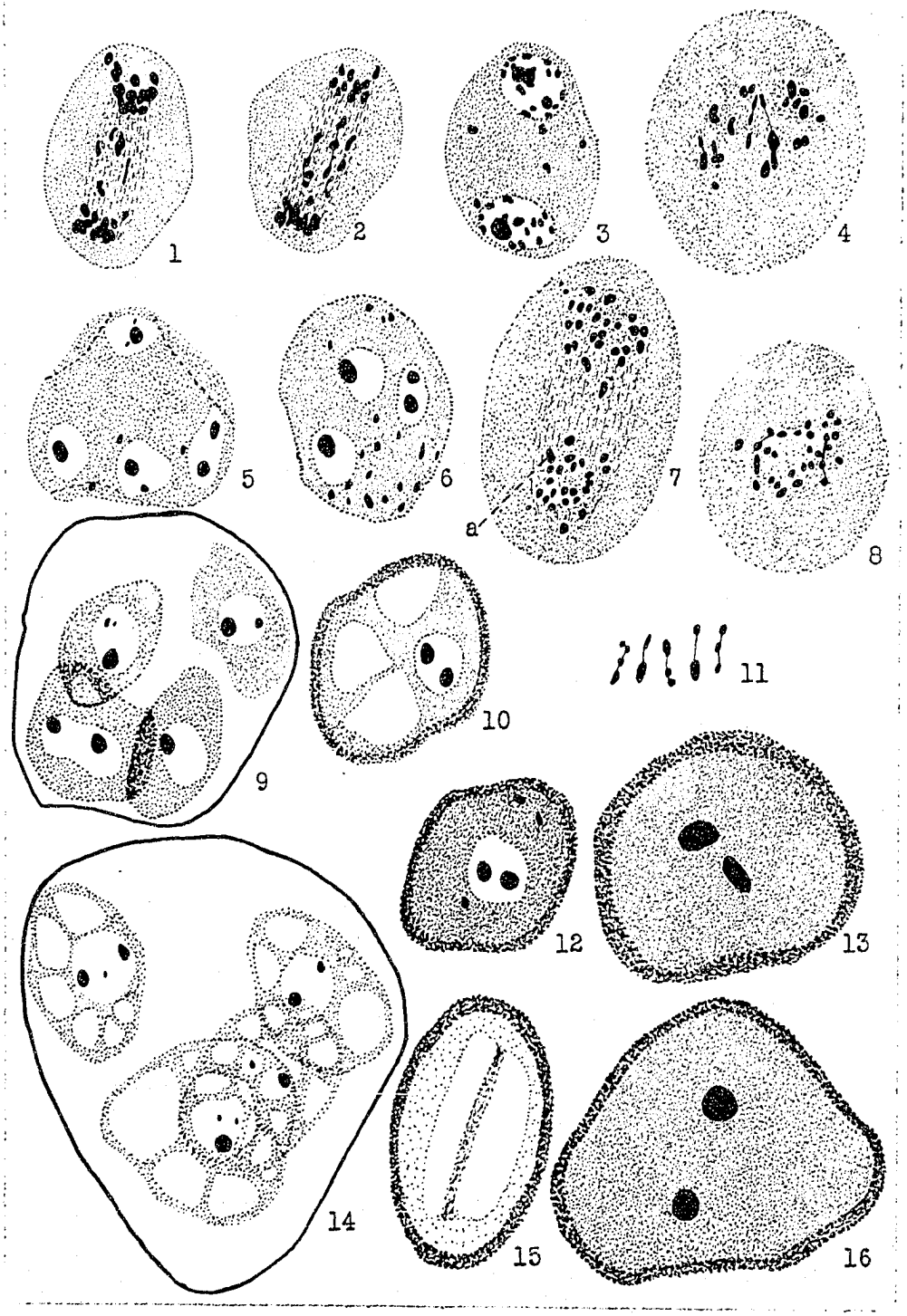
Fig. 13. Binucleate pollen grain.

Fig. 14. Vacuclated tetrads.

Fig. 15. Empty pollen grain.

Fig. 16. Typical binucleate pollen grain of the apple.

PLATE III



EXPLANATION OF PLATE IV
(Camera lucida drawings)
Magnification 4000x

Fig. 1. Anisim. Second metaphase showing one plate with 17 bivalents. The other plate is massed and uncountable.

Fig. 2. Anisim. First anaphase showing two sets of 17 univalent chromosomes. One set is in black, the other in outline.

Fig. 3. Starking. Second metaphase showing two plates, each with 17 chromosomes. Note the splitting of the bivalents for the anaphase separation.

Fig. 4. Stayman. First anaphase showing 20 chromosomes in one group and 27 in the other group.

Fig. 5. Stayman. Second metaphase showing 24 chromosomes in one plate. The other plate is uncountable.

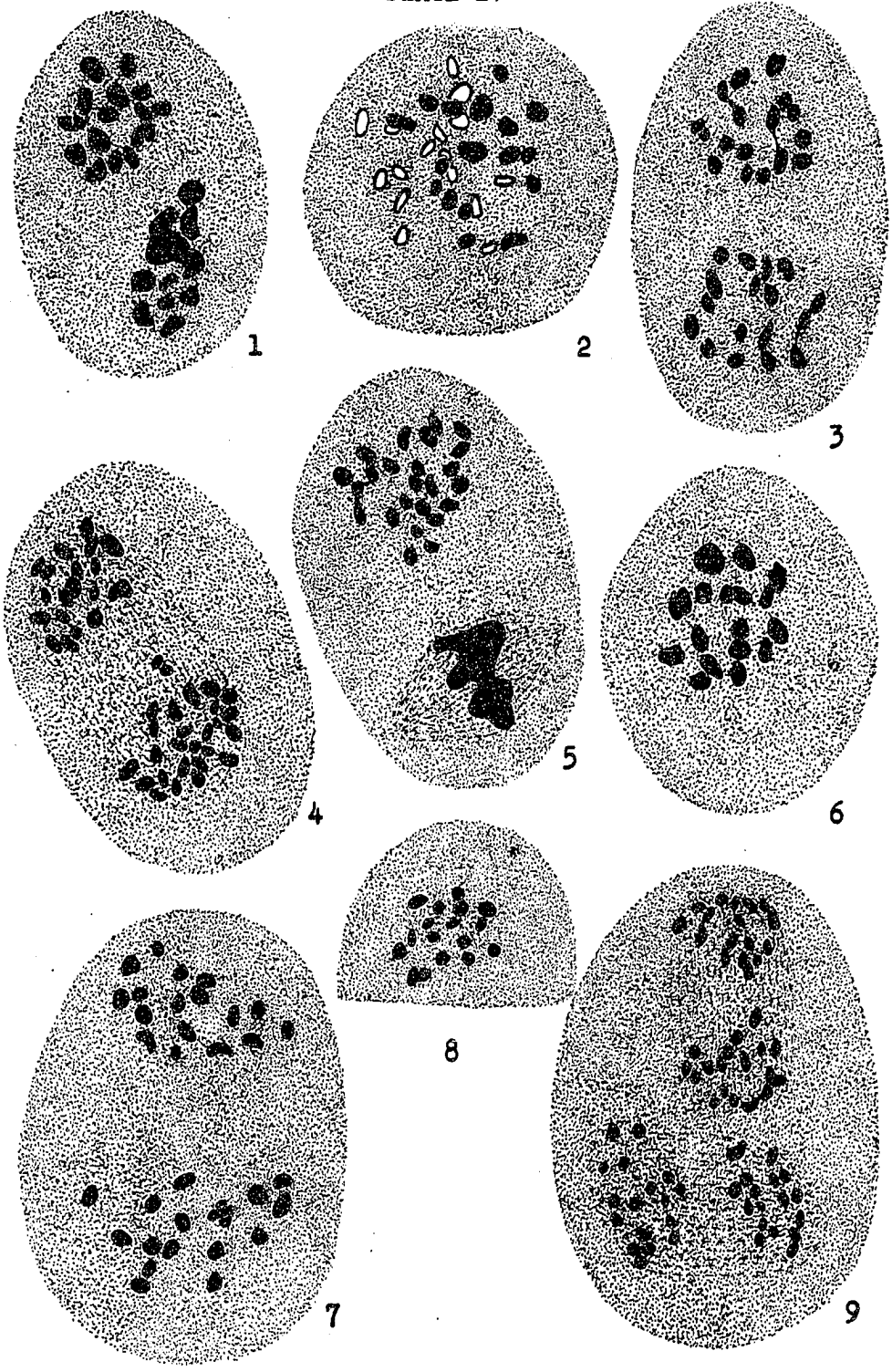
Fig. 6. Starking. First metaphase showing 17 bivalent chromosomes.

Fig. 7. King David. Second metaphase showing two sets of 17 bivalent chromosomes.

Fig. 8. Grimes. 1 figure from second metaphase showing 17 bivalents.

Fig. 9. Jonathan. Second anaphase showing four sets of 17 univalents.

PLATE IV



EXPLANATION OF PLATE V
(Photomicrographs 1450x except figures 16 and 17 which are 150x)

Fig. 1. Virginia Crab pollen mother cell showing lagging chromosomes at first anaphase.

Fig. 2. Virginia Crab pollen mother cell; first late telophase. Characteristic chromatin masses in the cytoplasm outside the newly formed nuclei.

Fig. 3. Virginia Crab pollen mother cell; second anaphase with lagging chromosomes.

Fig. 4. Hibernal pollen mother cell; first anaphase with numerous lagging chromosomes.

Fig. 5. Whitney pollen mother cell showing first anaphase.

Fig. 6. Whitney pollen mother cell; first anaphase with one slightly lagging chromosome.

Fig. 7. Whitney pollen mother cell; first telophase.

Fig. 8. Whitney pollen mother cell; normal second anaphase.

Fig. 9. Whitney pollen mother cell; normal second early telophase.

Fig. 10. Same as fig. 9 except that two nucleoli have developed in some nuclei at late second telophase.

Fig. 11. Three microspores released from normal Whitney tetrad.

Fig. 12. Whitney microspore with one nucleolus.

Fig. 13. Whitney microspore with two nucleoli.

Fig. 14. Whitney uninucleate pollen grain inside anther.

Fig. 15. Whitney binucleate pollen grain within the anther.

Fig. 16. Four Whitney pollen grains.

Fig. 17. "a" signifies the approximate location of the metaphase plate of the generative nucleus within the Whitney pollen tube.

Fig. 8. Whitney pollen mother cell; normal second anaphase.

Fig. 9. Whitney pollen mother cell; normal second early telophase.

Fig. 10. Same as fig. 9 except that two nucleoli have developed in some nuclei at late second telophase.

Fig. 11. Three microspores released from normal Whitney tetrad.

Fig. 12. Whitney microspore with one nucleolus.

Fig. 13. Whitney microspore with two nucleoli.

Fig. 14. Whitney uninucleate pollen grain inside anther.

Fig. 15. Whitney binucleate pollen grain within the anther.

Fig. 16. Four Whitney pollen grains.

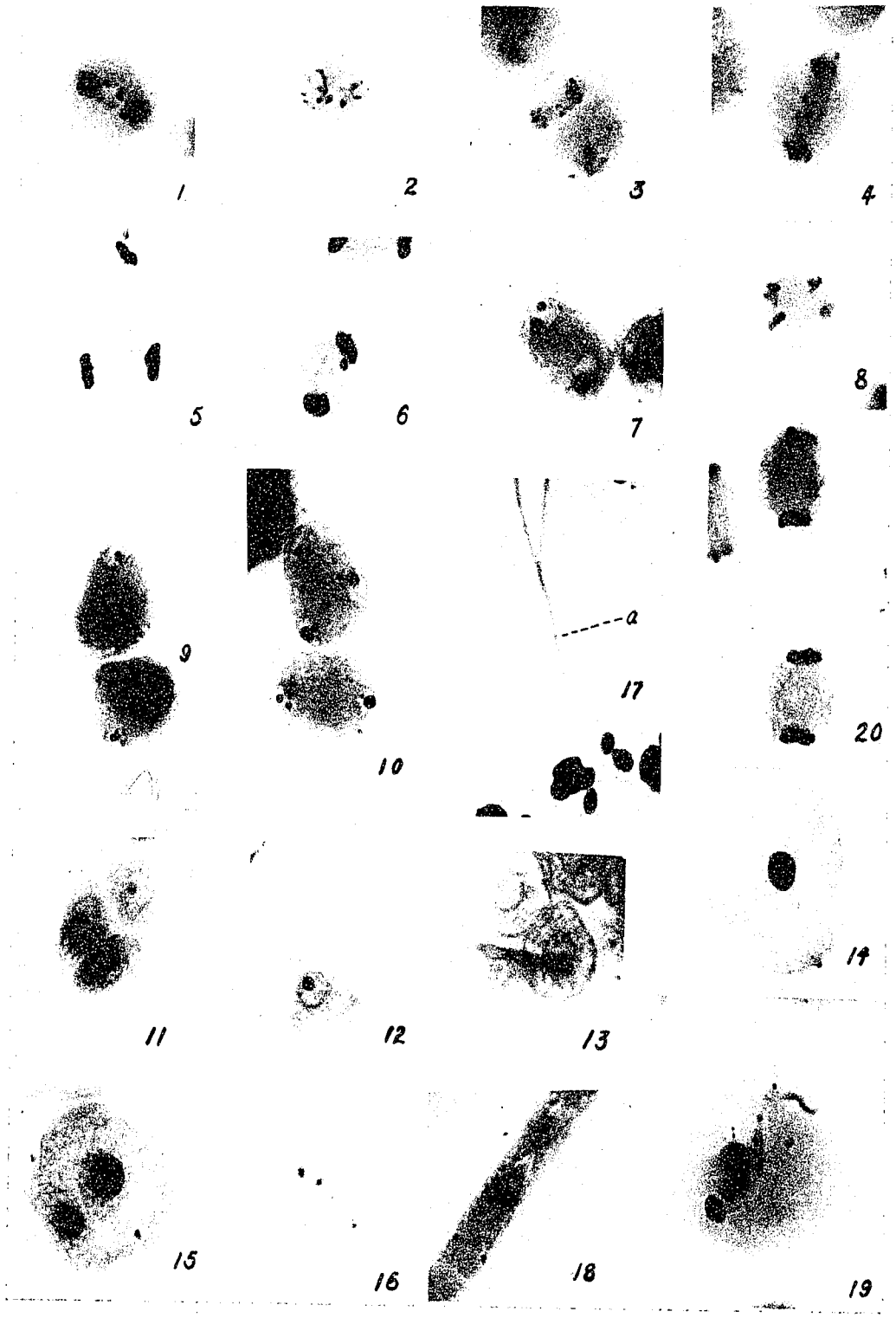
Fig. 17. "a" signifies the approximate location of the metaphase plate of the generative nucleus within the Whitney pollen tube.

Fig. 18. Enlargement of the area "a" showing a side metaphase view of the generative nucleus. Note the spindle arrangement.

Fig. 19. Normal mature pollen grain released from anther; note two nuclei and inclusions.

Fig. 20. Starking pollen mother cell; side view first anaphase.

PLATE V



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